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Complete Contents on First Inside Page

From Arcadi, p. 264

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TABLE OF CONTENTS

VOLUME 61 MARCH 1956 NUMBER 3

ORIGINAL ARTICLES

REGULAR DEPARTMENTS

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1. Shackman, N. H.; Heffer, E. T., and Kroop, I. G.: Am. Heart J. 48:509 (Oct.) 1954. • 2. Stollerman, G. H., and others: Am. J. Med. 15:645 (Nov.) 1953. • 3. McEwen, C.: M. Clin. North America 39:353 (March) 1955. • 4. Wood, H. F., and McCarty, M.: Am. J. Med. 17:768 (Dec.) 1954. • 5. McEwen, C., and Ziff, M.: M. Clin. North America (May) 1955, to be published.

A.M.A. Archives OF PATHOLOGY

Poliomyelitis

XV. The Spinal Cord

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An extensive literature has accumulated on the subject of the spinal cord changes in poliomyelitis. However, in spite of the numerous and comprehensive studies on this subject, there still remain many unanswered questions regarding the significance of the various pathologic changes as well as the correlation of such changes with the clinical symptomatology. Some of the many problems in spinal poliomyelitis that still warrant clarification are as follows: (1) the nature of the primary lesion; (2) the relationship of the meningitis to the underlying inflammatory spinal cord changes; (3) the relationship of the mesodermal-glial reaction to the neuronal damage; (4) the frequency of involvement of other cell groups aside from the anterior horn cells; (5) the frequency of spinal meningitis and its correlation with the spinal fluid changes and the tightness of the neck and back musculature, and (6) the actual correlation between the muscle paresis and the observable anterior horn cell damage. Because of these many unanswered questions, it would seem of definite value to review in detail the spinal cord pathology in a large series of cases in an attempt to arrive at some acceptable conclusions concerning these lesions and their clinical significance.

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From the Division of Neurology, University of Minnesota Medical School.

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REVIEW OF LITERATURE

Spinal poliomyelitis in children was first described in 1840, by Heine.[1] This was purely a clinical study and contained no anatomic verification. The first pathologic studies on this disease were made by Cornil,[2] who described the spinal cord in a 49-year-old woman who had been infected at the age of 2 years with a resultant atrophy of the limbs. Histologically no significant changes were observed. Duchenne[3] substantiated Cornil's findings. In a 14-month-old infant with paralysis, he found no changes in the anterior horn cells.

Atrophy of the anterior horn with disappearance of the nerve cells was first described by Prevost[4] in 1866 and Vulpian[5] in 1870. Charcot and Joffroy[6] in 1870 published the first detailed pathologic study in this disease. Their case was also one of chronic poliomyelitis. These authors carefully compared the regional muscle atrophy with the corresponding spinal cord levels and observed a definite and often severe decrease in the number of anterior horn cells. Secondary to the neuronal damage, there was also a mild glial increase. These authors were the first to emphasize the importance of the neuron damage and to demonstrate its accurate correlation with the clinical paralysis and the muscle atrophy. This concept of Charcot and Joffroy has continued to be popular and has received a large fol-

lowing (Parrot and Joffroy [7]; Leyden [8]; Schultze[9]; Turner[10]; Taylor[11]; Eisenlohr[12]; Sahli [13]; Déjerine [14]; Drummond [15]; Pette [16]; Fairbrother and Hurst [17]; Bodian and Howe [18]; Sabin [19]).

Many investigators not only supported the observations of Charcot but believed that the anterior horn cell damage was highly selective, implicating certain specific cell groups. Déjerine [14] believed that the anterior horn cell damage was limited to the inner group only. Horányi-Hechst [20] in a detailed study of 28 cases observed the severest damage in the cervical region and limited to the central cells. In the lumbosacral segments the lesions involved chiefly the middle groups, sparing for the most part, the lateral cell elements. Kawka [21] disagreed with this specificity of cell damage. He believed that if one studied serial sections of the spinal cord, different cell groups are damaged even at adjacent cord levels. Similar observations were made by Kohnstamm [22] and Goldscheider.[23]

Although the greatest emphasis in the literature has been on the anterior horn cell damage, it is apparent that other cell groups within the cord may be involved. Eisenlohr [12] in 1880 first described the extension of the spinal cord involvement to the small cells at the base of the posterior horn or to the internuncial cells. Similar damage to these small cells as well as to the posterior horn cells was subsequently reported (Turner [10]; Déjerine and Huet [24]; Taylor [11]; Allbutt [25]; Köhlisch, Lubarsch, and Smidt [26]; Mönckeberg,[27] and Stiefler and Schenk [28]). Stiefler and Schenk in a study of 12 cases observed the posterior horn cells to be involved in every case, although the degree of damage was not nearly so severe as that of the anterior horn cells. However, in the cases studied by Köhlisch and his associates and by Mönckeberg, the posterior horn cells were the chief cells implicated within the spinal cord. Wöhrmann [29] and Horányi-Hechst [20] described severe involvement of Clarke's columns. Horányi-Hechst reported on 28 cases; in 7 there was damage to Clarke's columns, and in 5, alterations within the lateral horn cells.

Many experimental investigations have been carried out in an attempt to determine the significance of the nerve cell damage as the primary lesions in spinal poliomyelitis. Fairbrother and Hurst [17] inoculated monkeys with poliomyelitis virus intracerebrally and systematically studied the spread of the virus by means of the histologic changes resulting within the various regions of the nervous system. They observed that the virus spread by means of the axons in a fairly systematic manner until it reached the midbrain. From the midbrain the virus spread indiscriminately by both motor and sensory axons, causing the disease to break out at many levels of the brain stem and cord. Owing to the greater susceptibility of the lumbar cord, these levels first manifested clinical paralysis. These authors admitted that meningitis and interstitial cell reaction always occurred, but they believed that the latter changes were always most marked where the nerve cell damage was severest and, therefore, were probably the result of such damage.

Bodian and Howe [18] in 1941 produced hemidecortication in monkeys, producing retrograde disappearance of nerve cells of the corresponding optic thalamus. When the virus was then inoculated directly into this thalamus devoid of nerve cells, very little mesodermal-glial reaction developed. From these studies the authors concluded that the mesodermal-glial response in poliomyelitis was secondary to a chemical resultant of the interaction of the virus with susceptible neurons rather than to the direct action of the virus upon mesodermal tissues.

Sabin [19] from a study upon human material also concluded that the primary lesion in poliomyelitis was the neuronal damage. He admitted that there was an associated interstitial and perivascular alteration. He was impressed by the fact that damaged neurons occurred among normal ones, indicating that the inflammatory process could not produce the nerve cell damage.

Although there is a great deal of work emphasizing the importance of neuronal dam-

age in spinal poliomyelitis, many investigators have taken issue with this concept and have been more impressed by the importance of the inflammatory changes in this disease. As early as 1871 Roger and Damaschino [30] took issue with this primary neuronal theory. These investigators studied three cases of acute poliomyelitis which terminated fatally from 2 to 13 months after the onset of the illness. They were impressed by the severe inflammatory changes involving chiefly the anterior horns and often extending throughout the gray matter. The involved regions showed vascular changes and hemorrhages, perivascular and diffuse leucocytic infiltrations, and even tissue-softening with cavitation. Ganglion cell damage occurred, but usually in those areas most severely injured. These authors believed that poliomyelitis was an interstitial myelitis with the nerve cell alterations being secondary to the inflammatory reaction.

Roth [31] in 1873 substantiated the findings of Roger and Damaschino. Within the anterior horns of an acute case of poliomyelitis Roth observed scattered areas of cavitation with numerous perivascular and diffuse infiltrates. He stated positively that poliomyelitis was an interstitial myelitis and that the neuronal damage was secondary.

Similar emphasis has been placed upon the inflammatory reaction in poliomyelitis by Turner,[10] Goldscheider,[23] Peabody and his associates,[32] Wickman,[33] Rissler,[34] Wöhrmann,[29] and Környey.[35] Rissler's studies, published in 1888, have been unsurpassed for their accuracy and detail and remain today as a histopathologic classic. He studied the lesions in five cases, three acute and two chronic. He described in great detail both the ganglion cell changes and the interstitial cell reactions. The former he studied from the earliest stages of swelling and chromatolysis to the final phases of destruction with neuronophagia, fragmentation, and dissolution. Almost every type of mesodermal reaction was observed and commented upon. He described vascular congestion, hemorrhage, perivascular changes, diffuse leucocytic reactions, gliosis, tissue injury, and even cavitation. Rissler was puzzled as to which of the alterations were primary. He admitted that both occurred together, but he believed that he could more commonly observe neuronal changes without interstitial reactions than the reverse.

In contrast to this view were those of Goldscheider,[23] Wickman,[33] Peabody,[32] Wöhrmann,[29] and Környey.[35] Goldscheider studied only a single case but was greatly impressed by the inflammatory changes. He was convinced that poliomyelitis was an acute myelitis, and that the nerve cell changes were secondary to the inflammatory process. Wickman was even more insistent in his views. He studied five cases carefully and was impressed by the mesodermal reactions that seemed to localize within the gray matter of the spinal cord. This author believed that the severity of the ganglion cell change usually was in direct ratio to the intensity of the interstitial reaction. He insisted that degenerated nerve cells unaccompanied by interstitial changes never occurred in man.

Peabody and his associates believed that the primary process in poliomyelitis was a perivascular infiltration around the pial and spinal blood vessels. They believed that in many cases this cellular exudate was so severe that it completely compressed the lumen of the vessel, producing secondary changes within the nerve cells. They admitted that some neuron damage could also be caused directly by the virus.

Környey reported on seven cases of poliomyelitis in which death occurred in from 1 to 11 days, and all patients were extremely paralyzed. He was impressed by the fact that the earliest alteration observed in the spinal cord was an infiltration of inflammatory elements, which appeared within the first day of the illness. Actual changes in the anterior horn cells did not occur until the third day. He also noted that the mesodermal changes were much more widespread than the nerve cell alterations, and that generally there was no correlation between the two.

In 1935 Horányi-Hechst reported on 38 cases of acute poliomyelitis ranging in age from 2 months to 29 years. He again described the characteristic lesions in poliomyelitis, but for the first time offered an adequate correlation and interpretation of the findings in this disease. His deductions certainly warrant listing at the present time and were as follows:

1. The nerve cell changes were the most important change, since upon them depends the functional damage.

2. The inflammatory mesodermal reactions often formed the dominating change and were frequently present in areas where no nerve cell damage had occurred or in areas where there were no nerve cells, such as in the molecular layer of the cerebellum. He believed, therefore, that these interstitial changes were also primary changes and were produced by the same agent and independent of the neuronal alterations.

3. In every single case, regardless of the acuteness of the illness, some mild or severe meningitis appeared. The meningitis also showed no correlation with the other cellular changes and occurred where nerve cells were uninvolved.

The significance of the meningitis in spinal poliomyelitis has engaged the interest of investigators long before the publications of Horányi-Hechst. Dauber [26] in 1893 first emphasized the importance of meningeal involvement in poliomyelitis. Since his work, a large number of investigators have been impressed by the meningeal changes in this disease (Harbitz and Scheel [27]; Wickman [28]; Abramson [38]; Wöhrmann [29]). Harbitz and Scheel studied 17 cases, 13 acute and 4 chronic. In serial sections of the spinal cord, they believed that foci of inflammation in the cord always corresponded with pial involvement. They believed that the process started in the pia and extended along the perivascular spaces into the spinal cord.

Wickman was also impressed by the meningeal involvement in this disease. He observed the most intense pial changes in the lumbosacral region, where the entire cord was often surrounded by inflammatory elements which were usually perivascular. He observed that the earliest spinal cord changes consisted of an inflammatory reaction around the vessels in the region of the entrance of the central vessels into the cord at the bottom of the anterior commissure. The veins were more severely involved than the arteries, and the infiltrate, no matter how intense, appeared to be limited to the region of the blood vessels.

Wöhrmann substantiated the findings of Wickman. He, too, emphasized the involvement of the central artery in the anterior commissure and the extension of this perivascular infiltration into the adjacent spinal cord tissue.

Spielmeyer [39] and Swan [40] both agreed that the meninges were involved in spinal poliomyelitis but did not believe that this meningitis had any correlation with the associated myelitis. Spielmeyer reported a number of cases in which spinal cord changes had occurred in the absence of any changes within the meninges. He insisted that poliomyelitis was a primary disease of the central nervous system, and not of the meninges. Swan in a more recent publication substantiated the observations of Spielmeyer. In a study of eight cases he could not find any correlation between the meningeal changes and the inflammatory alterations within the spinal cord. He, too, believed that this disease was a primary myelitis, but that the inflammatory changes did not extend inward from a primary involvement of the meninges.

In view of the relative frequency of the meningeal involvement in spinal poliomyelitis, one would anticipate that the spinal rootlets and ganglia might also be implicated. Reports of such involvement, however, are not too frequent. The first mention of spinal ganglia injury was by Forssner and Sjövall.[41] Pette [16] also described a lymphocytic infiltration of the ganglia in isolated cases. In 1890 Williamson [42] reported a marked destruction of the anterior roots in a single patient, who died suddenly with a quadriplegia. Between the destroyed fibers were many inflammatory elements. The posterior rootlets in this case were intact. Wickman [28] in his studies also described a mild involvement of the anterior roots with some infiltration of inflammatory elements between the fasciculi of the anterior rootlets. Staemmler [43] in a report on four

cases gave a detailed description of the changes within the spinal ganglia. He observed a lymphocytic infiltration throughout the ganglia. Many of the ganglion cells were destroyed with fragmentation and chromatolysis. Often the capsule around the cells were proliferated.

There has been considerable discussion regarding the nature of the inflammatory elements in poliomyelitis. Most of the older investigators believed that the infiltrates were composed chiefly of the leucocyte or its developmental forms (Turner [10]; Siemerling [44]; Rissler [34]; Redlich [45]; Harbitz and Scheel [37]). Harbitz and Scheel believed that most of these interstitial cells consisted of lymphocytes and plasma cells. Wickman also believed that these inflammatory elements were leucocytic in origin. He believed that the large mononuclear cells that appeared in the later stages of the illness were polyblasts derived from the lymphocytes.

Other authors strongly disagreed with the observations of Wickman and insisted that these large mononuclear cells were derived from the fixed cells. Strauss [46] believed that they came from the adventitial cells of the blood vessels. Goldscheider [23] emphasized their origin from glial elements as well as from endothelial elements of the blood vessels. Glial origin of these cells was also proposed by Schröder,[47] Wallgren,[48] and Homen.[49] Häuptli [50] made a special study of these elements using the oxidase reaction and believed that his observations proved conclusively that most of these large mononuclear cells are derived from the glial elements.

PRESENT INVESTIGATION

The entire spinal cord was available in 50 cases of fatal bulbar poliomyelitis. In these cases the changes in all other areas of the nervous system had been studied and were available for comparison with the alterations observed within the spinal cord (Baker, Matzke, and Brown [51]; Matzke and Baker [52]; Matzke and Baker [53]; Baker, Cornwell, and Brown [54]; Baker, Cornwell, and Tichy [55]; Baker and Cornwell [56]). Blocks were taken from all levels of the spinal cord and prepared with the Nissl stain, the hematoxylin and eosin stain, and Weil's stain. The following four types of histopathologic changes were observed within the spinal cord: (1) a meningeal inflammation, (2) a diffuse and/or focal interstitial cell reaction, (3) neuronal damage, and (4) focal necrosis.

MENINGEAL INVOLVEMENT

Involvement of the spinal meninges is a moderately common occurrence in poliomyelitis and was present in 50% of our cases. Of our 50 cases, 17 (34%) showed active inflammatory changes in the meninges of the cervical cord, oftenest in the midcervical region; 12 cases (24%) showed meningeal changes in the thoracic cord, oftenest at the high thoracic levels, and 17 cases (34%) had active meningeal infiltration at some level of the lumbosacral cord, chiefly at the upper and midlumbar levels (Table 1). Fourteen cases revealed meningeal inflammation at only a selected cord level, whereas most of the cases manifested some meningeal change in at least two separate areas, usually the cervical and lumbar regions.

In none of these cases was the meningitis present alone, but it was usually associated with severe inflammatory changes within the spinal cord as well as with intense nerve cell damage. The meningeal involvement was never very severe and did not compare in intensity with the severity of the associated myelitis. Invariably, when meningeal changes were observed, there was an associated myelitis at the corresponding spinal cord level. There appeared to be no direct association between the meningeal changes and the neuronal damage, and in only one case were these two changes present alone without the accompanying inflammatory cord alterations (Table 1).

The meningeal changes consisted primarily of an infiltration of the leptomeninges and the subarachnoid space with moderate numbers of lymphocytes, larger mononuclear cells, occasional polymorphonuclear leucocytes, and very occasional erythrocytes (Fig. 1). The number of inflammatory ele-

TABLE 1.—*Number of Cases Showing Meningitis, Myelitis, and Neuron Damage in Fifty Unselected Cases of Bulbar Poliomyelitis*

	Cervical Cord		Thoracic Cord		Lumbosacral Cord	
	No.	Per Cent	No.	Per Cent	No.	Per Cent
Meningitis plus myelitis	2	4	2	4	8	16
Meningitis, myelitis, and neuron damage	15	30	10	20	8	15
Myelitis alone	4	8	7	14	7	14
Neuron damage alone	0	0	2	4	1	2
Myelitis and neuron damage, no meningitis	27	54	28	56	23	46
Meningitis alone	0	0	0	0	0	0
No changes	2	4	1	2	2	4
Meningitis and neuron damage	0	0	0	0	1	2
Total, 50 cases						
Meningitis with or without other pathology	17	34	12	24	17	34
Necrosis	9	18	8	16	6	12

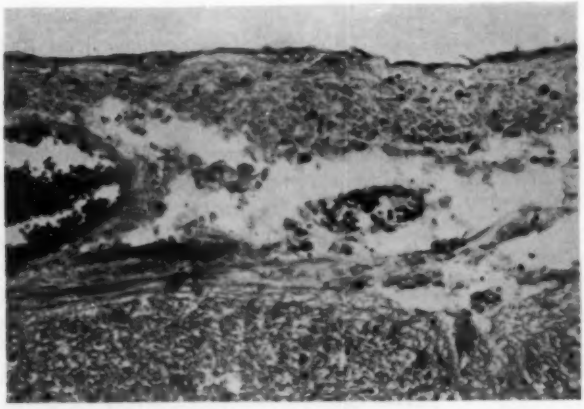

Fig. 1.—Mild meningeal reaction. There is a definite thickening of the leptomeninges. Hematoxylin and eosin stain.

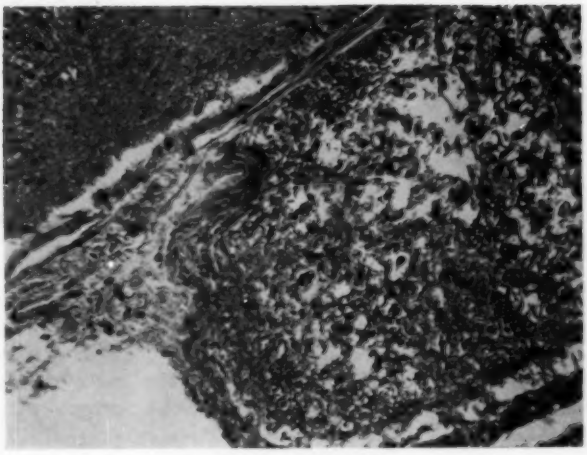

Fig. 2.—Compression of the anterior rootlet in the lumbosacral region by the involved leptomeninges. There is a beginning vacuolation and fragmentation of the rootlet fibers. Hematoxylin and eosin stain.

ments never attained marked proportions and did not compare in intensity with the severe changes within the spinal cord of the same cases. The commonest site of involvement was anteriorly in the region of the anterior commissure. This was the only site of involvement in five cases. In this region there was often a perivascular mononuclear-cell cuffing about the anterior spinal vessels, which could often be followed into the substance of the cord. Meningeal involvement in the posterior area was never seen as an isolated occurrence but did occasionally occur in association with a diffuse meningeal inflammation. In three cases the meningeal changes were severest in the anterolateral regions.

Fig. 3.—Extensive inflammatory changes limited to the anterior horn. There is some extension of the process to the base of the posterior horn. Hematoxylin and eosin stain.

Eight cases showed a definite thickening of the leptomeninges, even though the inflammatory changes were not too severe. This thickening usually involved the arachnoid and, to a slighter degree, the pia (Fig. 1). It was not present uniformly throughout all levels of the spinal cord but involved the cervical levels in three cases, the thoracic levels in four cases, and the upper lumbar segments in one case. In six cases this meningeal thickening was of sufficient intensity to cause at least a mild degree of anterior root compression with some demyelination of the anterior root fibers (Fig. 2).

A careful survey of the meningeal changes in our cases of poliomyelitis would indicate that this involvement probably is not the primary change in this disease. Invariably the meningeal changes appeared to be associated with and dependent upon intense mesodermal glial reactions within the associated spinal cord segments. Certainly these findings would suggest that the spinal cord changes were probably the primary ones, and that the milder meningeal changes were secondary to the changes within the cord. This conclusion is further strengthened by the fact that there were many cases with severe spinal cord inflammation in which no meningitis was observed (Table 1).

Mesodermal-Glial (Interstitial) Reaction

These alterations comprise the predominant tissue change and often occur together with severe neuronal damage. Mild-to-severe parenchymal inflammation was observed in at least some level of the spinal cord in each of the 50 cases of fatal poliomyelitis. Of the cases studied, 96% showed inflammation at some level of the cervical and thoracic cord, with 92% at some level of the lumbosacral cord. In 84% of the cases all levels of the spinal cord revealed inflammatory alterations (Table 1).

The inflammatory pathology is located primarily in the anterior gray horn and, less frequently, in the lateral horn, the base of the posterior horn, and in the white matter immediately adjacent to the anterior horns (Figs. 3 and 4). Perivascular collections of mononuclear cells comprise one of the

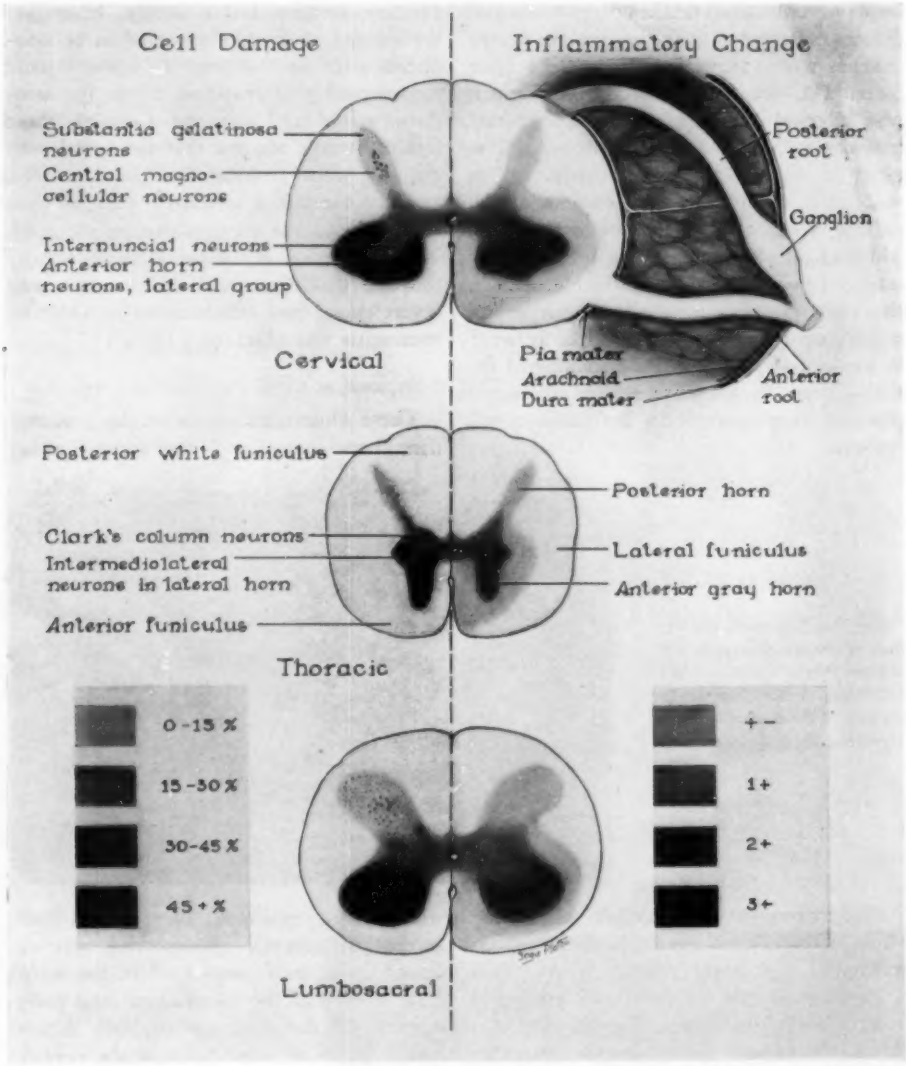

Fig. 4.—Intensity of inflammatory and nerve cell changes within the various regions of the spinal cord. The severity of the tissue changes are demonstrated by the comparative darkness of the shaded areas.

most constant changes seen. The degree of involvement varies from a few scattered cells that incompletely surround the vessel to heavy layers of leucocytes which fill the perivascular spaces and even invade the outer vascular tissues. Usually the cells are predominantly small lymphocytes. Occasionally, in acute fulminating cases, a few polymorphonuclear leucocytes are observed interspersed among the other cells. This type of vascular change first appears in two regions of the cord, namely, within the anterior horns and in the white matter adjacent to the anterior commissure. Although often occurring simultaneously, these changes may appear independently in each location.

After a few days numerous vessels became involved throughout the spinal cord.

When the perivascular infiltration is very severe, the leucocytes may extend inward to involve the vessel wall or outward to invade the adjacent tissue. When the extension is inward, there results a definite irritation to the vessel, producing an endothelial swelling and proliferation as well as some connective-tissue increase within the wall itself. In either event the vessel lumen becomes narrowed, occasionally producing a secondary anemia and tissue necrosis.

Extension of leucocytes from the vessel into the adjacent tissues occurs only in the severer cases and usually predominates within the gray matter of the anterior horns. In many cases the leucocytic invasion remains localized, although varying greatly in size. Within the larger foci the underlying tissues undergo a softening and fragmentation, eventually being invaded by phagocytes. In many cases the leucocytic infiltration spreads diffusely throughout the gray, and even the adjacent white, matter. Even within these areas of diffuse infiltration, there can also be seen additional focal and perivascular collections, which usually predominate within the regions of the anterior horns.

In evaluating our cases for the degree of inflammatory changes within the spinal cord we listed the intensity of the changes as ranging from ± to 3+. The criteria which was used for each degree of inflammatory involvement was as follows (Fig. 4):

±: Small foci of inflammatory cells of less than 100μ in diameter. The perivascular cuffing is predominantly mononuclear and loosely packed and averages two cells or less in thickness.

1+: Slightly larger foci of approximately 200μ in diameter. The perivascular cuffing is heavier and averages about four cells in thickness. The diffuse inflammatory process is not intense and implicates not more than one-half of a given anterior horn.

2+: Inflammatory foci are large and often exceed 500μ in diameter. The perivascular cuffs are very thick and often contain six to eight cells in thickness. The diffuse inflammation is intense, implicating the complete anterior horn and the adjacent white matter.

3+: Same as above, except that there is an associated destruction of the underlying tissue resulting in an inflammatory necrosis.

Using the above method of evaluation, 58% of our cases showed only a ± to 1+ involvement, while 20% of the cases had a 3+ involvement with actual areas of necrosis within the anterior horns, sometimes extending into the lateral horns in the thoracic levels.

The inflammatory changes within the spinal cord usually showed a very diffuse distribution throughout all levels of the spinal cord, with the cervical and upper thoracic segments showing the most consistent alterations. Scattered involvement of certain segments with a skipping of adjacent areas was almost never observed.

Bleeding occurs only in the most fulminating cases. The hemorrhages usually are in the form of petechiae of a ball type. Eighteen of our cases showed petechiae associated with the inflammatory changes.

Neuronal Alterations

Normal Structure.—The principal cellular groups within the spinal cord are the anterior horn cells, the internuncial cells, the posterior horn cells, the intermediolateral cell column, and Clarke's columns. The normal structure of most of these cell groups has been well documented. It might be well, however, to summarize briefly the normal appearance of these different elements in order to describe the criteria which we used for cell damage.

1. Anterior Horn Cells: These cells are very uniform in structure, though varying in size and distribution throughout the length of the spinal cord. Variation in size is present even at a single level, but the majority have a long diameter of somewhat over 100μ and a transverse diameter of 30μ to 60μ. The cells are multipolar and possess large, clearly demarcated, frequently angular Nissl granules (Fig. 5*A*). The cells are artificially divided, with much overlapping,

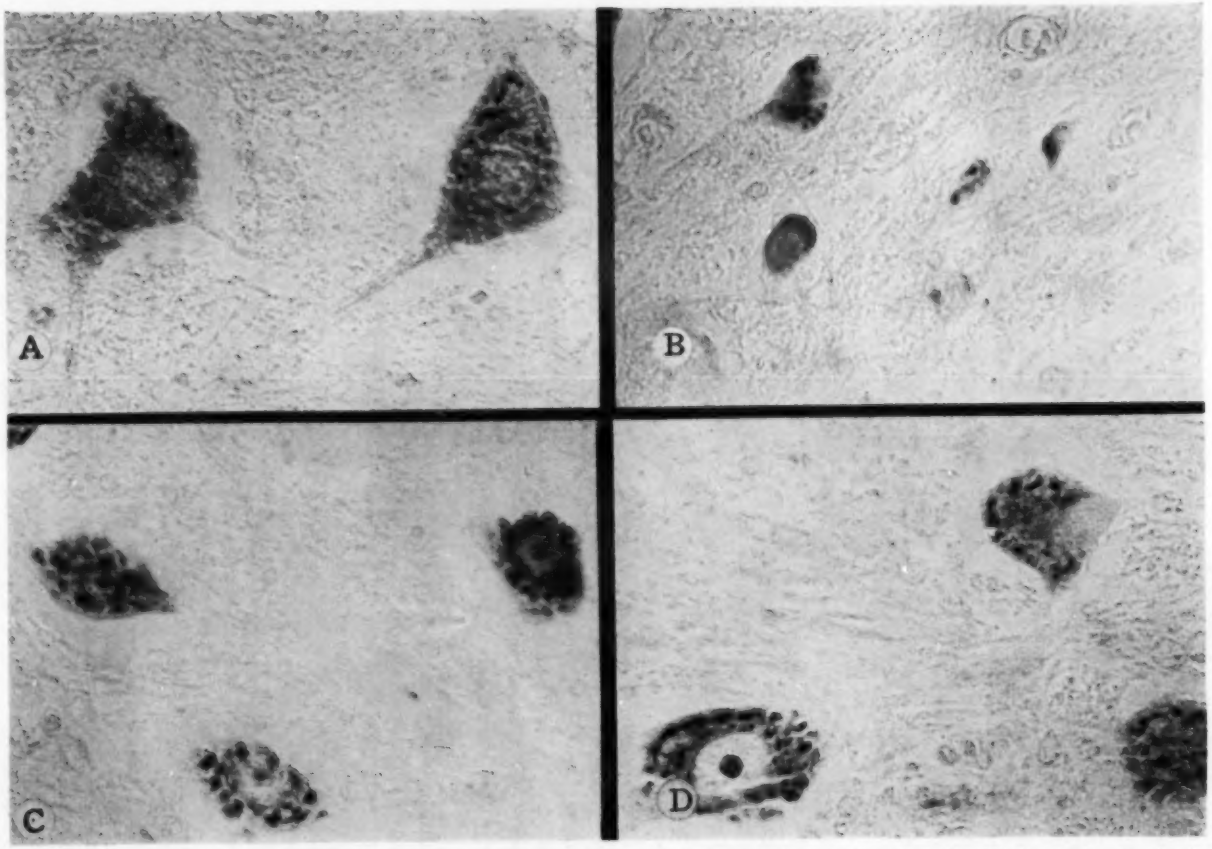

Fig. 5.—Nissl stain. *A*, normal anterior horn cells. The architecture of these cells is well defined and the Nissl granules are clearly demarcated. *B*, normal internuncial cells from the base of the posterior horns. These are small irregular elements with poorly demarcated rounded Nissl granules and round or oval nuclei. They averaged 10μ to 20μ. *C*, normal cells of the intermediolateral cell column. These cells average 12μ to 45μ and have large vesicular nuclei and well-demarcated Nissl bodies. *D*, normal cells of Clarke's columns. These cells resemble the anterior horn cells of the spinal cord.

into two medial columns, two lateral columns, and a central group.

2. Internuncial Cells: These cells are rather diffusely distributed throughout the gray matter of the spinal cord, with no definite grouping except for those cells situated in the posterior central portion of the anterior horn and extending into the base of the posterior horn (posteromarginal group). There is no basic difference between the structure or distribution of these cells at different cord levels, although the number of cells varies considerably from level to level. The largest concentration of internuncial cells is present in the cervical enlargement (C5 to C8), and the second greatest number is present in the lumbosacral enlargement (L2 to L4). These cells are small ovoid, spindle, or multipolar neurons, usually with a maximum diameter of 10μ to 20μ. They contain unevenly spaced, poorly demarcated rounded Nissl granules varying from fine to moderately coarse in size. The nuclei of these cells are usually round or oval and may be eccentric in position (Fig. 5*B*).

3. Posterior Horn Cells: The posteromarginal nucleus covers the surface of the posterior horn, especially the apex, with a layer of large cells which are spindle-shaped, pyramidal, or polygonal in form. These cells have a central round nucleus and poorly defined Nissl granules that are irregular in shape and size. These cells are most numerous in the lumbosacral cord and are relatively few in the thoracic segments. They are regarded as association neurons of the posterior gray column and were never observed to be damaged in our cases of poliomyelitis.

The gelatinous substance of Rolando underlies the posteromarginal cells and consists of numerous small cells, ovoid or polygonal in form with a large central round or oval nucleus and very fine Nissl substance. This group of cells extends the entire length of the spinal cord and is regarded as the chief sensory nucleus of the spinal cord for afferent impulses.

A few larger "central magnocellular cells" of spindle or polygonal form with large Nissl granules lie central to the gelatinous substance and share its function in part.

4. Intermediolateral Cell Column: The larger neurons lying in the lateral gray horn of the spinal cord and the junctional area between the anterior and posterior horns may be divided into the medial and lateral sympathetic nuclei. The lateral nucleus actually consists of several adjacent cell columns, with the most lateral or superior portion constituting the intermediolateral column or lateral horn. This apical group extends from the caudal part of the eighth cervical to the second or third lumbar spinal cord segment. These neurons are ovoid, spindle-shaped, or, less often, multipolar in shape and range in size from 12μ to 45μ in their longest diameter. They contain large central vesicular nuclei and relatively fine, though variable in size, and poorly demarcated Nissl bodies (Fig. 5*C*).

5. Clarke's Column Cells: This cell column is situated in the medial portion of the base of the dorsal horn. It extends from the lower cervical region through the thoracic and upper lumbar segments, being most prominent in the lower thoracic levels where there are about 15 to 20 cells in an average microscopic section. The cells are often as large as the anterior horn cells. They are ovoid or spherical in shape with a large vesicular and often eccentrically placed nucleus. The Nissl granules are prominent and coarse and are usually situated at the periphery of the cell body (Fig. 5*D*).

Pathologic Studies.—By far the most consistent neuronal damage in spinal poliomyelitis is to the anterior horn cells. Fortunately the normal structure of these cells is so well-defined that even mild alterations are detectable on histologic study. Slight structural alterations can also be evaluated for many of the other large cell groups within the spinal cord such as the intermediolateral cells and Clarke's column cells. Pathologic alterations within the smaller

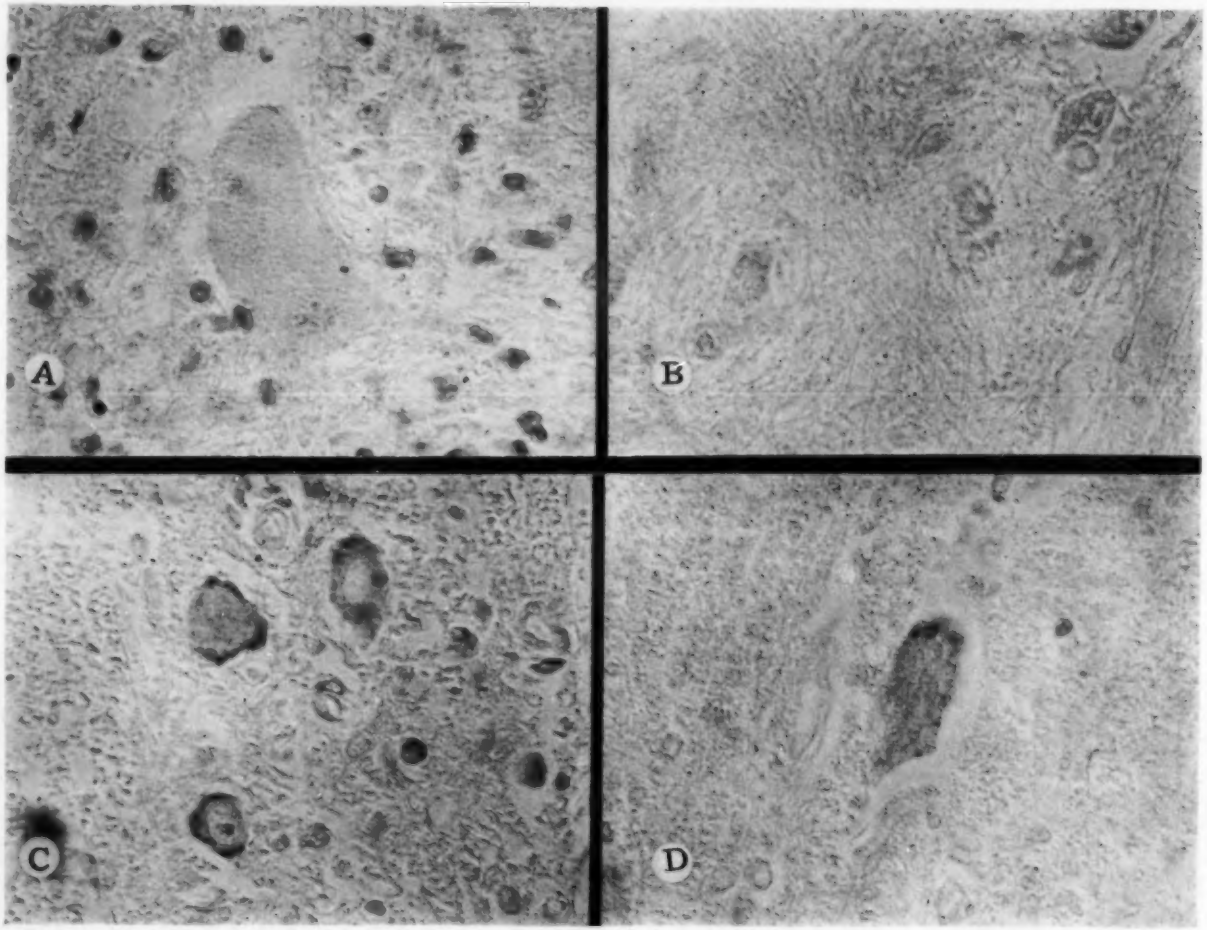

Fig. 6.—Nissl stain. *A*, anterior horn cell in acute poliomyelitis showing marked swelling and complete chromatolysis. *B*, complete destruction of internuncial cells. Only a faint outline of the original cells can be seen. *C*, complete and perinuclear chromatolysis of the intermediolateral cells in fatal bulbar poliomyelitis. *D*, complete chromatolysis of a cell of Clarke's column in acute poliomyelitis.

cells such as the internuncial cells and the posterior horn elements are much more difficult to evaluate, and only extreme alterations can be accepted as criteria for cell damage. Because of the marked differences in the normal cell structure, it seems advisable to describe those alterations which were considered by us as abnormal for each of the different spinal cord cell groups.

1. Anterior Horn Cells: The earliest alterations in the anterior horn cells consist of an acute swelling and a diffuse perinuclear chromatolysis (Fig. 6*A*). At this stage the process is still reversible, and if the disease becomes arrested, many of these cells will regain their normal function. If the process continues, the cell processes become fragmented and detached, leaving a rounded, swollen, light-staining cell that is identified chiefly by the still intact nucleus. Later even the nucleus undergoes changes, becoming eccentrically placed, losing its staining properties, and eventually showing fragmentation, pyknosis, and extrusion from the cell. Many of these severely damaged neurons undergo fragmentation and shrinkage, leaving only an irregular mass of cytoplasm as a remnant of the original nerve cell. The stage of cell alteration at which neuronophagia occurs will depend upon the rapidity of the neuronal destruction by the virus. In many cases apparently structurally intact cells will become invaded by phagocytes, indicating that the cell, although appearing healthy, has been destroyed by the virus. Neuronophagia is not the usual process, and most of the ganglion cells that are destroyed undergo fragmentation and dissolution without neuronophagia.

The anterior horn cell damage often shows a most unusual distribution. The virus apparently has a selective action and picks out scattered elements throughout the spinal cord. Isolated ganglion cells will frequently show changes in a setting of many normal elements. On the other hand, in severe cases most of the anterior horn cells will show changes of varying degree and severity, with only scattered cells being entirely uninvolved and undamaged.

In our cases the severest anterior horn cell damage occurred in the cervical levels, where 96% of the cases showed significant alterations (Table 2). The commonest alteration consisted of total destruction of the cells, although chromatolysis, swelling, ghost-cell formation, and fragmentation were also observed (Fig. 6*A*). In 7 cases over 80% of all the anterior horn cells were destroyed, whereas the average over-all destruction for all 50 cases was 38%. The severest cell damage within the cervical cord occurred in the middle and lower cervical levels, though often all levels were equally involved. The cervical anterior horn cell damage was usually diffuse, involving both medial and lateral cell groups. In six cases the medial groups of cells were spared.

TABLE 2.—*Different Cell Groups Involved in the Spinal Cord of Each of the Fifty Cases of Bulbar Poliomyelitis*

	Cervical Cord		Thoracic Cord		Lumbosacral Cord	
	No.	Per Cent	No.	Per Cent	No.	Per Cent
Anterior horn cell alone	31	62	15	30	21	42
Anterior horn cell and internuncial cell	17	34	7	14	7	14
Anterior horn cell and intramedial lateral cells	..	..	8	16	..	..
Anterior horn cell and Clarke's columns	..	..	..	..	1	2
Anterior horn cells, internuncial cells, and intramedial lateral cells	..	..	4	8	..	..
Anterior horn cells, internuncial cells, and posterior horn cells	..	..	..	..	1	2
Anterior horn cells, internuncial cells, intermedial lateral cells, and Clarke's columns	..	..	1	2	..	..
Internuncial cells alone	..	..	3	6	2	4
Posterior horn cells alone	..	..	..	..	..	..
Intramedial lateral cells alone	..	..	2	4	..	..
Internuncial cells and posterior horn cells	..	..	..	..	2	4
No cell involvement	2	4	10	20	16	32

In the thoracic cord 70% of the cases showed anterior horn cell damage (Table 2). As in the cervical cord, total destruction of the cells was commonest, although many cells showed slighter degrees of injury. Cell damage was slightly commoner and severer at the upper thoracic levels and least intense in the lower thoracic cord. No sparing of any of the cell groups was noted, although the percentage of damage was somewhat greater in the medial group.

The anterior horn cell damage was least intense in the lumbosacral cord, where 60% of our 50 cases showed involvement. In this cord level an average of 30% of the cells were injured. The severity and frequency of the cell damage graded off as one descended the spinal cord, with the cell damage least intense at the low sacral levels (Table 2).

2. Internuncial Cells: Because of the marked variation in the normal structure of these cells, evaluation of neuronal pathology was very difficult, and extremely rigid criteria had to be set up in order to be sure that actual cell damage was being evaluated. Only three types of alterations were accepted as being abnormal; complete chromatolysis with ghost-cell formation, fragmentation of the nucleus and cell body, and total destruction with marked reduction in the number of cells (Fig. 6*B*).

By these standards 17 of our 50 cases, or 34%, had an average of 24% of the internuncial cells damaged in the cervical region; 15 cases, or 30%, had an average of 32% of these cells altered in the thoracic region; and 10 cases, or 20%, showed an average of 33% of the cells damaged at the lumbosacral levels. The most extensive involvement seemed to occur in the posteromarginal group of cells situated in the posterior aspect of the anterior horn and at the base of the posterior horn (Table 2).

3. Posterior Horn Cells: The same criteria were used to establish cell damage as were used for the internuncial cells, namely, complete chromatolysis, fragmentation, or total cell destruction. Any milder type of cell change was not considered reliable because of the normal variation in the structure of these elements. Because of these rigid criteria, many injured cells no doubt were overlooked, and our figures for involvement of this nuclear group are probably very conservative.

With use of these rigid criteria definite damage to these cells was observed in only one fatal case. In this case 20% of the cells in the upper and midlumbar cord were destroyed in comparison with 80% of anterior horn cells and 20% of the internuncial cells, which were also involved at the same cord level of this same case.

4. Intermediolateral Cell Group: These cells are large with well-defined architecture. It is, therefore, relatively easy to detect even minor cell alterations. The criteria used were similar to those described for the anterior horn cells. The earliest changes consisted of partial to complete chromatolysis and cell swelling. Severer damage manifested itself by fragmentation, nuclear damage, ghost-cell formation, and complete dissolution of the cells (Fig. 6*C*).

This cell group was involved in 15, or in 30%, of our cases. In two cases it was the only cell group implicated, all other neuronal elements being spared (Table 2). In almost all cases the damage occurred bilaterally, and the cells were involved to some extent throughout the length of this cell column. There was a tendency for the cell damage to be severer in the upper and midthoracic cord levels.

5. Clarke's Columns: These cells are relatively large cells with well-defined structural elements. Even minor alterations in cell structure can easily be recognized. The same criteria were used for determining cell damage as was used for the intermediolateral cells and the anterior horn cells (Fig. 6*D*).

This cell group was damaged in only two cases (Table 2). In one case over 50% of these cells were destroyed, and there was an associated involvement of all cell groups within the same cord segments. In the second case about 30% of the nucleus dorsalis cells

were damaged in the uppermost lumbar segments and this process was associated with changes within the anterior horn cells.

As can be seen in Table 2, by far the most severely damaged cell groups within the spinal cord were the anterior horn cells and the internuncial elements within the cervical and lumbosacral cord segments. Involvement of the remaining neuronal elements occurred relatively infrequently and was always associated with some involvement of the anterior horn cells or the internuncial cells.

Neuron damage alone occurred infrequently. Invariably such involvement was associated with fairly intense inflammatory changes. In a number of cases inflammatory changes were present in the absence of any nerve cell alteration. This was much commoner than the isolated occurrence of nerve cell changes.

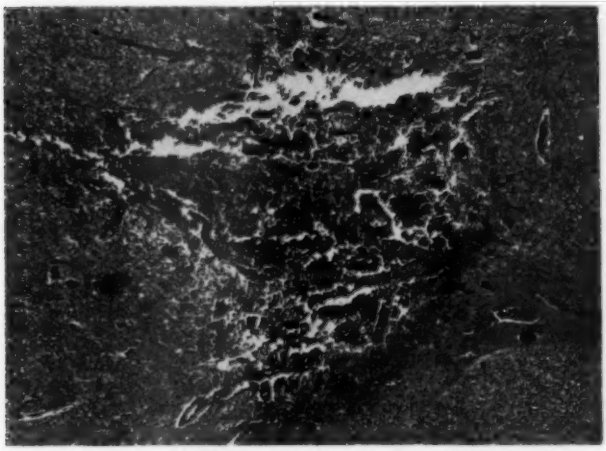

Fig. 7.—Large area of inflammatory necrosis within the anterior horn in acute poliomyelitis. The underlying tissues are undergoing fragmentation. Hematoxylin and eosin stain.

Necrosis

Parenchymal softening is not a constant feature of poliomyelitis. It occurs chiefly in the severer cases and is usually secondary either to a vascular occlusion or to an extensive exudative reaction. Most commonly parenchymal softening is observed within areas of severe exudative changes. Since such severe inflammatory alterations occur almost exclusively within the gray matter of the cord, areas of tissue necrosis are observed almost exclusively in such regions (Figs. 3 and 7). The involved tissues undergo a softening and even a fragmentation. Macrophages soon invade the necrotic area to become intermixed with the numerous leucocytes already present. These macrophages phagocytize the necrotic tissue as well as the destroyed leucocytes, eventually clearing the entire area of debris. The end-result, observed only in chronic cases of poliomyelitis, consists of numerous irregular cavities scattered irregularly throughout the anterior horn and body of the spinal cord gray matter. Occasionally these cavitations are multilocular and extensive enough to replace completely the involved gray matter and even to extend into the adjacent white matter. Curiously enough, this destructive process stimulates very little glial reaction. It is unusual, therefore, to observe any glial replacement or walling-off of any of these cavitations, even in long-standing chronic processes.

The epidemics of poliomyelitis in 1946-1949 in Minnesota were very severe, and an unusual number of cases developed inflammatory necrosis of the spinal cord with resulting cavitation. Since most of the patients died during the acute illness, the necrotic lesions contained a large number of inflammatory elements with a minimal amount of phagocytes. Areas of necrosis

confined to the anterior horns were observed in 13 cases, of which 3 were at the cervical levels, 5 at the thoracic levels, 4 at the lumbar levels, and 1 at the sacral level. In the majority of these cases the necrotic lesions were bilateral. In five cases the necrosis was localized to the lateral portion of the gray matter, whereas in five cases it was more centrally placed. In two cases the necrosis was so extensive that it destroyed the entire anterior horns and extended into the base of the posterior horns.

as well-documented histopathologic observations. Unfortunately, most of our material was obtained from acute bulbar deaths which occurred during a large epidemic of poliomyelitis. Because of the large number of acutely ill patients being cared for at the time, the staff often was too busy or the patient too ill to allow for a careful evaluation of muscle involvement. Most of these patients were placed in respirators shortly after admission, and because of the severity of the illness no opportunity arose to recheck

TABLE 3.—*Correlation of Muscle Involvement and Neuronal Damage*

No.	Duration of Illness, in Days	Upper Limb Involvement	Cervical Cord Neuron Damage	Lower Limb Involvement	Lumbosacral Cord Neuron Damage	Chest Involvement	Thoracic Cord Neuron Damage
1	2	Paresis	30% ant. horn cell	None	None	Intercostal paralysis 4+	40% ant. horn cell
2	9	Paresis	55% ant. horn cell	Paresis	40% ant. horn cell	Intercostal paralysis 4+	55% ant. horn cell 10% internunc. cell
3	2	Paralysis	55% ant. horn cell	Paralysis	30% ant. horn cell	None	None
4	1	Paralysis	90% ant. horn cell	Paralysis	30% ant. horn cell	Intercostal paralysis 4+	80% ant. horn cell 15% internunc. cell
5	..	None	10% ant. horn cell	None	None	None	None
6	4	Paralysis	70% ant. horn cell 40% internunc. cell	Paralysis	50% ant. horn cell 25% internunc. cell	Intercostal paralysis 4+	80% ant. horn cell 60% internunc. cell
7	2	Paresis	90% ant. horn cell 30% internunc. cell	Paralysis	90% ant. horn cell 70% internunc. cell	Intercostal and diaphragm paralysis 4+	80% ant. horn cell
8	2	Paresis	20% ant. horn cell	None	None	Intercostal and diaphragm paralysis 4+	40% ant. horn cell
9	..	None	None	Paralysis	70% ant. horn cell	Diaphragm 2+	20% ant. horn cell
10	3	None	10% ant. horn cell	None	10% ant. horn cell	None	None
11	2	Paresis	50% ant. horn cell	None	None	Intercostal paresis 2+	40% ant. horn cell
12	5	Paralysis	30% ant. horn cell	None	None	Intercostal paralysis 4+	75% ant. horn cell
13	2	None	15% ant. horn cell 15% internunc. cell	None	None	None	None
14	4	Paralysis	70% ant. horn cell	Paralysis	30% ant. horn cell	Intercostal paralysis 4+	90% ant. horn cell
15	2	Paresis	20% ant. horn cell	None	None	Intercostal and diaphragm paralysis 4+	40% ant. horn cell

CLINICAL CONSIDERATIONS

Clinical Correlation with Neuron Damage.—As early as 1870 Charcot [6] pointed out the careful correlation between the degree of muscle paralysis and the anterior horn cell damage in the corresponding levels of the spinal cord. Generally it has been assumed that the clinical muscle involvement should be dependent upon the degree of neuronal damage within the spinal cord. In order to make such a clinical-pathologic study, it is imperative that one have available very careful evaluation of the degree and extent of muscle involvement as well

the development of paralysis of the limbs. In spite of this obvious defect in our clinical information, in 31 of our 50 cases there was a very close correlation between the muscle involvement and the damage to the anterior horn and/or internuncial cells of the corresponding cord segments. Fifteen of these cases have been listed in Table 3 in order to show the nature of this correlation. It can be seen from this Table that up to 18% of the anterior horn cells can be destroyed without any apparent clinical muscle weakness. As a rule, when up to 40% of the neurons are involved, definite paresis of the

corresponding muscles results. When over 40% of the anterior horn cells are damaged, paralysis usually ensues.

The internuncial cells seem to play very little role in the resulting muscle involvement. In most of our cases the muscle involvement correlated with the anterior horn cell damage and was present, even in the absence of any internuncial cell injury. The degree or nature of the muscle weakness apparently was not altered by the associated damage of the corresponding internuncial cells.

In 12 of our cases there appeared to be a gross discrepancy between the clinical muscle involvement and the neuronal damage. These cases are listed in Table 4. It is readily apparent that the greatest disagreement (10 out of the 12 cases) was in the absence of recorded muscle weakness in cases with severe neuronal damage. As stated previously, this discrepancy probably is due to a deficiency in the clinical studies. Most of these patients were placed in respirators shortly after admission, and because of the severity of the illness no attempt was then made to follow the development of muscle weakness. Many of these patients could have developed severe paralysis of the limbs prior to death without such paralysis being detected or recorded by the staff.

In isolated cases it is possible that the anterior horn cells may have been severely destroyed in a short segment of cord without resulting in paralysis, because of the fact that most muscles derive their innervation from several cord levels and their functional capacity is thus endowed with a large factor of safety. Because of the many cord sections studied histologically by us, we do not feel that this factor played a very important role in the discrepancies observed in our clinicopathological studies.

In seven of our cases the illness was so fulminating that no clinical data were available for comparison with the pathologic studies.

TABLE 4.—*Absence of Correlation Between Muscle Involvement and Neuronal Damage*

No.	Duration of Illness, in Days	Arm Involvement	Cervical Cord Neuron Damage	Leg Involvement	Lumbosacral Cord Damage	Chest Involvement	Thoracic Cord Damage
1	5	None	85% ant. horn cell	None	95% ant. horn cell	None	80% ant. horn cell
2	5	None	55% ant. horn cell	None	10% ant. horn cell	Intercostal and diaphragm paralysis 4+	25% ant. horn cell
3	4	None	60% ant. horn cell	Paresis 2+	None	Intercostal paralysis 4+	30% ant. horn cell
4	1	None	40% ant. horn cell 10% internunc. cell	Paresis 2+	30% ant. horn cell 20% internunc. cell	Intercostal paralysis 4+	80% ant. horn cell
5	..	None	45% ant. horn cell	None	None	None	20% ant. horn cell 30% internunc. cell
6	..	None	50% ant. horn cell	None	20% ant. horn cell	None	None
7	22	Paresis 2+	10% ant. horn cell	None	70% ant. horn cell	None	30% ant. horn cell
8	3	Paresis 1+	None	None	None	Intercostal paralysis 2+	None
9	3	None	60% ant. horn cell	None	10% ant. horn cell	Intercostal paralysis 4+	60% ant. horn cell
10	..	None	65% ant. horn cell	None	10% ant. horn cell	None	15% ant. horn cell
11	6	None	45% ant. horn cell	Paralysis 4+	10% ant. horn cell	Intercostal paralysis 4+	10% ant. horn cell
12	2	None	50% ant. horn cell	Paralysis 4+	70% ant. horn cell 15% internunc. cell	Intercostal paralysis 2+	20% ant. horn cell 15% internunc. cell

Clinical Correlation with Inflammatory Changes.—In 37 of our cases adequate laboratory studies were available, so that these could be correlated with the clinical findings and pathologic studies (Table 5). The spinal fluid cell count ranged from 13 to 1208. In 28, or 72%, the cell count varied from 50 to 500. As seen in Table 5, there was no correlation between the spinal fluid pleocytosis and the clinical or pathologic findings. Severe tightness of the neck and back muscles was often present in the presence of only a few cells in the spinal fluid, and was occasionally absent even when the pleocytosis was fairly marked. However,

when the spinal fluid cell count was elevated over 200, the patient generally manifested considerable tightness of the neck and back muscles. The spinal fluid in poliomyelitis has been studied by many investigators (Hyland and co-workers [57]; Abramson [38]; Peabody and co-workers [32]; Eyre-Brook,[58] and Drury and Sladden [59]). Most of these investigators reported findings very similar to ours. The spinal fluid cell count was rarely increased to over 500 cells. As in our cases, the greatest cell increase invariably occurred during the first week of the illness and appeared to be somewhat more prominent in patients with paralysis (Drury and Sladden,[59] and Abramson [38]). Hyland and his associates also reported a greater pleocytosis in patients with paralysis; however, in two patients without paralysis, there occurred the highest counts of 1250 and 2500 cells.

Since this tightness of the neck and back muscles is a very constant and characteristic feature of acute poliomyelitis, an attempt was made to correlate these findings with the pathological changes observed within the meninges and spinal cord. As seen from Table 5, no such correlation was possible. The tightness of these muscles does not appear to be dependent upon either the alterations within the meninges or the spinal cord.

TABLE 5.—*Correlation of Inflammatory Changes with Clinical Symptoms and Signs*

No.	Age	Stiff Neck or Back Muscles	Spinal Fluid: Total Count	Spinal Fluid: %, PMN	Duration of Illness, in Days	Meningitis	Cord Changes, Inflammatory
1	8	3+	13	9	..	...	±
2	25	...	17	24	6	...	±
3	12	3+	22	38	..	...	±
4	31	2+	46	45	7	...	1+
5	27	3+	70	81	3	2+	1+
6	11	...	110	18	9	...	±
7	14	3+	175	31	4	...	±
8	14	...	284	74	17	1+	2+
9	22	2+	300	28	5	...	2+
10	17	2+	495	9	1	1+	±
11	28	3+	600	96	5	...	2+
12	15	3−	1,208	95	6	...	1+

COMMENT

At this time it might be well to reconsider many of the unanswered questions concerning spinal poliomyelitis which were raised at the onset of this paper in order to determine whether some of these questions might now be answered in view of our studies in these 50 cases.

What is the nature of the primary lesion? From our studies it would appear that the virus of poliomyelitis possesses an affinity for nervous tissue in general, but for no elements of these tissues in particular. The constancy with which the meninges, blood vessels, interstitial elements, and neurons are affected indicate that they all react to the presence of the virus. Certainly, as our studies reveal (Table 1), any one of these elements in selected areas or in selected cases may be primarily or even exclusively involved, this process emphasizing their basic susceptibility in this illness. Naturally, only involvement of the neurons within the spinal cord would produce functional damage and clinical disturbances.

What is the relationship of the meningitis to the underlying inflammatory spinal cord changes? Although some degree of meningitis was present at some cord level in 50% of our cases, this inflammatory process was very mild and at no time equaled the intense interstitial cell changes within the spinal cord. In most cases the meningitis consisted of scattered mononuclear cells collected within the leptomeninges in the region of the anterior commissure and surrounding the anterior spinal vessels. This process was never extensive enough to cause compression or occlusion of these vessels. In only an oc-

casional case did there appear to be any possibility of extension of the inflammatory processes along these spinal vessels into the adjacent brain tissue. When one considers the usual intensity of the inflammatory process within the spinal cord which often produced an actual inflammatory necrosis, one would suspect that if any spread did occur, it would have to be from the spinal cord to the meninges. Certainly no case of meningitis was seen in which there were no severe inflammatory changes within the spinal cord at that same level.

Is there any relationship between the inflammatory changes and the neuronal damage within the cord? Although in most instances these two types of tissue alterations occur together, particularly in the severer cases, one still feels that they probably are independent of one another. Certainly in our cases (Table 1) a number of instances were seen in which, at different levels of the spinal cord, either the myelitis or the neuron changes occurred alone. In those cases with severe inflammatory necrosis the neurons within the damaged area would also be involved. Only in such cases can one feel that there would be a direct causal relationship between these two tissue changes.

Are cell groups besides the anterior horn cells implicated in this disease? Although the anterior horn cells are by far the most frequently damaged in poliomyelitis, all types of spinal cord neurons may be involved. One of the most commonly ignored cell groups in the spinal cord is the internuncial cell group. These small elements are scattered throughout the gray matter at all levels of the spinal cord. In poliomyelitis these small neurons are severely damaged in almost one-third of the cases (Table 2). Since these cells are rarely injured alone but usually have some accompanying anterior horn cell damage, it is a little difficult to determine just what clinical manifestations such damage would produce.

The cells of the intermediolateral cell column are also frequently injured in poliomyelitis. This cell group was involved in 30% of our cases. The damage to this cell group might well account for many of the autonomic disturbances that result in this illness. Certainly this must be true in those cases in which only the spinal cord is involved without any associated implication of the higher autonomic centers in the brain stem and hypothalamus.

The remaining cell groups such as the cells of the posterior horn and of Clarke's column are only rarely implicated in this illness. In any severe case with extensive destruction of large areas of the spinal cord, one might expect these cell groups to be included in the pathologic process. However, as a selective process by the virus, these groups are generally by-passed, with damage being limited to the anterior horn cells, the internuncial cells, and the cells of the intermediolateral cell column.

Is there any correlation between the pathologic meningitis, the tightness of the neck and back muscles, and the spinal fluid changes? Tightness of the neck and back muscles is one of the most striking clinical manifestations of spinal poliomyelitis. The etiology of these findings has, as yet, not been determined. It can be assumed that they may be secondary to rootlet irritation from an associated meningeal involvement. If this were the case, the degree of muscle tightness should correlate with the pathologic changes within the meninges as well as with the spinal fluid cell count. No such correlation was observed in our studies (Table 5). In many cases with intense tightness of the neck and back muscles, there were no pathologic changes within the meninges, and the spinal fluid cell count was low. Our studies do not offer an adequate explanation of this striking clinical finding. One might suspect that the damage to some of the higher centers may well play a role in producing these marked functional changes within the neck and back musculature. However, any attempt to expand on such an explanation must remain in the field of speculation.

How accurately can one correlate the muscle weakness with the anterior horn cell damage? In spite of the fact that our clinical findings in many of the more severely ill

patients were inadequate, there appeared to be a very specific correlation between the degree of anterior horn cell damage and the muscle weakness. It would appear from our studies that up to 20% of these neurons could be destroyed before weakness of the musculature would become apparent. When over 40% of the cells were damaged, almost complete paralysis resulted. Certainly the cord segments involved played a very important role in the degree of muscle weakness, since many muscles receive a nerve supply from many segments of the cord. In 12 of our 40 cases there was a definite discrepancy in the anterior horn cell damage and the degree of muscle weakness. In most of these cases the discrepancy arose primarily in the failure to record muscle weakness even when the spinal cord was severely injured. It was believed that this discrepancy was due to an inadequacy of the clinical observations. Most of these patients were acutely ill with bulbar poliomyelitis and were in a respirator. All efforts were directed toward keeping these patients alive, and in many no attempt was made to reevaluate muscle weakness once the patient was placed in the respirator.

CONCLUSIONS

A detailed histologic study of the entire spinal cord was undertaken in 50 cases of bulbar poliomyelitis, and the pathologic alterations were correlated with the clinical symptoms.

Meningeal involvement occurred in 50% of our cases. This meningeal involvement was invariably mild and was usually present in the anterior commissure, often surrounding the spinal vessels. It bore little correlation with the inflammatory or neuronal changes within the underlying spinal cord tissues. There was also no correlation between the degree of meningeal change and the clinical manifestations, such as the tightness of the neck and back muscles or the spinal fluid cell count.

All cell groups were damaged in our cases, the anterior horn cells, the internuncial cells, and the cells of the intermediolateral cell column being the most severely involved. Anterior horn cells were damaged in 86% of our cases, the internuncial cells in 24%, and the intermediolateral cell column in 30%. The cells of the posterior horn and of Clarke's columns were only involved in isolated cases, and such involvement appeared to be secondary to the associated severe involvement of the entire spinal cord.

Damage to the anterior horn cells correlated fairly well with the degree of muscle weakness. Since the internuncial cells were rarely involved alone, it was impossible to correlate their damage with any type of clinical manifestation.

REFERENCES

1. Heine, J.: Beobachtungen über Lähmungszustände der unteren Extremitäten und deren Behandlung, Stuttgart, F. H. Köhler, 1840.

2. Cornil, V.: Paralysie infantile: Autopsie; Altérations de la moelle épinière, des nerfs et des muscles, Compt. rend. Soc. biol. **5**:187, 1863.

3. Duchenne (de Boulogne) fils: De la Paralysie atrophique graisseuse de l'enfance, Arch. gén. méd. **2**:28, 1864.

4. Prevost, J. L.: Observation de paralysie infantile: Lésion des muscles et de la moelle, Compt. rend. Soc. biol. **2**:215, 1866.

5. Vulpian, A.: Cas d'atrophie musculaire graisseuse datant de l'enfance: Lésions des cornes antérieures de la substance grise de la moelle épinière, Arch. physiol. norm. et path. **3**:316, 1870.

6. Charcot, J.-M., and Joffroy, A.: Cas de paralysie infantile spinale avec lésions des cornes antérieures de la substance grise de la moelle épinière, Arch. physiol. norm. et path. **3**:134, 1870.

7. Parrot, J., and Joffroy, A.: Note sur un cas de paralysie infantile, Arch. physiol. norm. et path. **3**:309, 1870.

8. Leyden, E.: Beiträge zur pathologischen Anatomie der atrophischen Lähmungen der Kinder und der Erwachsenen, Arch. Psychiat. **6**:271, 1875.

9. Schultze, F.: Beiträge zur Pathologie und pathologischen Anatomie des centralen Nervensystems, insbesondere des Rückenmarkes: Zur Lehre von der spinalen Kinderlähmung und der analogen Lähmung der erwachsenen (poliomyelitis acuta anterior), Arch. path. Anat. **68**:128, 1876.

10. Turner, F. C.: A Portion of the Spinal Cord with Drawings and Microscopical Specimens, from a Case of Acute Anterior Poliomyelitis in a Child, Fatal Within 6 Weeks from the Onset, Tr. Path. Soc. London **30**:202, 1879.

11. Taylor, F. C.: Spinal Cord from a Case of Infantile Paralysis, Tr. Path. Soc. London **30**:197, 1879.

12. Eisenlohr, C.: Einige Lähmungsformen spinalen und peripheren Ursprungs: Zur Pathologie und pathologischen Anatomie der spinalen Kinderlähmung, Deutsches Arch. klin. Med. **26**:557, 1880.

13. Sahli, H.: Zur Lehre von den spinalen Localisationen; Sectionsbefund bei einer alten Kinderlähmung mit eng localisirter Atrophie, Deutsches Arch. klin. Med. **33**:360, 1883.

14. Déjerine, J.: Note sur 2 cas de paralysie infantile, Bull. Soc. anat. Paris **53**:130, 1878.

15. Drummond, D.: On the Nature of the Spinal Lesion in Poliomyelitis Anterior Acuta, or Infantile Paralysis, Brain **8**:14, 1886.

16. Pette, H.: Über experimentelle Poliomyelitis, Deutsche Ztschr. Nervenh. **116**:163, 1930.

17. Fairbrother, R. W., and Hurst, E. W.: Pathogenesis of, and Propagation of the Virus in, Experimental Poliomyelitis, J. Path. & Bact. **33**: 17, 1930.

18. Bodian, D., and Howe, H. A.: Neurotropism and the Genesis of Cerebral Lesions in Poliomyelitis: An Experimental Study, Bull. Johns Hopkins Hosp. **68**:58, 1941.

19. Sabin, A. B.: Pathology and Pathogenesis of Human Poliomyelitis, J. A. M. A. **120**:506, 1942.

20. Horányi-Hechst, B.: Zur Histopathologie der menschlichen Poliomyelitis acuta anterior, Deutsche Ztschr. Nervenh. **137**:1, 1935.

21. Kawka, V.: Beiträge zur pathologischen Anatomie der spinalen Kinderlähmung, Halle, C. A. Kaemmer & Co., 1889.

22. Kohnstamm, O.: Bericht über die Schnittserien-Untersuchung eines Falles von spinaler Kinderlähmung (als Anhang zu der vorstehenden Abhandlung von Dr. A. Goldscheider über Poliomyelitis), Ztschr. klin. Med. **23**:556, 1893.

23. Goldscheider, A.: Über Poliomyelitis, Ztschr. klin. Med. **23**:556, 1893.

24. Déjerine, J., and Huet, E.: Contribution à l'étude de la paralysie atrophique de l'enfance à forme hémiplégique, Arch. physiol. norm. et path. **1**:375, 1888.

25. Allbutt, C.: Infantile Paralysis, Lancet **2**: 83, 1870.

26. Köhlisch, H.; Lubarsch, O., and Smidt, H.: Pathologie der spinalen Kinderlähmung, Ergebn. allg. Path. u. path. Anat. **16**:1, 1912.

27. Mönckeberg, J. G.: Anatomischer Befund eines Falles von Landryschem Symptomenkomplex, München. med. Wchnschr. **50**:1958, 1903.

28. Stiefler, G., and Schenk, E.: Klinische und anatomische Beobachtungen über die Heine-Medin'sche Krankheit und deren Beziehungen zur Economoschen Krankheit, Deutsche Ztschr. Nervenh. **130**:68, 1933.

29. Wöhrmann, W.: Über Poliomyelitis anterior acuta, Arch. path. Anat. **259**:446, 1926.

30. Roger, H., and Damaschino, F. T.: Recherches anatomo-pathologiques sur la paralysie spinale de l'enfance, Compt. rend. Soc. biol. **3**:49, 1871.

31. Roth, M.: Anatomischer Befund bei spinaler Kinderlähmung, Arch. path. Anat. **58**:263, 1873.

32. Peabody, F. W.; Draper, G., and Dochez, A. R.: Clinical Study of Acute Poliomyelitis, Monograph 4, Rockefeller Institute for Medical Research, New York, 1912.

33. Wickman, I.: Acute Poliomyelitis (Heine-Medin Disease), translated by W. J. M. A. Maloney, Nervous and Mental Disease Monograph 16, New York, Nervous and Mental Disease Publishing Company, 1913.

34. Rissler, J.: Zur Kenntniss der Veränderungen des Nervensystems bei Poliomyelitis anterior acuta, Nord. med. ark. **20**:1, 1888.

35. Környey, S.: Die Bedeutung der mesodermalen Reaktion und der Systemelektivität in der Pathologie der Poliomyelitis, Ztschr. Neurol. u. Psychiat. **146**:724, 1933.

36. Dauber: Zur Lehre von der Poliomyelitis anterior acuta, Deutsche Ztschr. Nervenh. **4**:200, 1893.

37. Harbitz, F., and Scheel, O.: Akute Poliomyelitis und verwandte Krankheiten: Pathologisch-anatomische Untersuchungen aus den Epidemien in Norwegen, 1903-1906, Deutsche med. Wchnschr. **33**:1992, 1907.

38. Abramson, H. L.: Spinal Fluid in Poliomyelitis and Its Differentiation from Fluids of Other Infections, Am. J. Dis. Child. **10**:344, 1915.

39. Spielmeyer, W.: Zur Histopathologie und Pathogenese der Poliomyelitis, Ztschr. Neurol. u. Psychiat. **142**:159, 1932.

40. Swan, C.: Anatomical Distribution and Character of the Lesions of Poliomyelitis, with Special Reference to Type of Cell Affected and to Portal of Entry of Virus, Australian J. Exper. Biol. & M. Sc. **17**:345, 1939.

41. Forssner, G., and Sjövall, E.: Über die Poliomyelitis acuta samt einem Beitrag zur Neuronophagienfrage, Ztschr. klin. Med. **63**:1, 1907.

42. Williamson, R. T.: Early Changes in the Spinal Cord in Acute Anterior Poliomyelitis of the Adult, Med. Chron. **12**:454, 1890.

43. Staemmler, M.: Die Spinalganglien und Rückenmarkswurzeln bei Poliomyelitis und Polyneuritis, Klin. Wchnschr. **27**:56, 1949.

44. Siemerling, E.: Zur pathologischen Anatomie der spinalen Kinderlähmung, Arch. Psychiat. **26**: 267, 1894.

45. Redlich, E.: Beitrag zur pathologischen Anatomie der Poliomyelitis anterior acuta infantum, Wien. klin. Wchnschr. **7**:287, 1894.

46. Strauss, I.: The Pathology of Acute Poliomyelitis, Nervous and Mental Disease Monograph 6, New York, Nervous and Mental Disease Publishing Company, 1910.

47. Schröder: Encephalitis and Myelitis: Zur Histopathologie der Kleinzelligen Infiltration im Nervensystem, Monatsschr. Psychiat. u. Neurol. **43**:146, 1918.

48. Wallgren, A.: Zur Kenntnis der feineren Histologie und Pathogenese der Heine-Medinschen Krankheit, Berl. klin. Wchnschr. **51**:937, 1914.

49. Homen, E. A.: De l'action du streptocoque et de ses toxines sur les nerfs, les ganglions spinaux et la moelle spinère, Compt. rend. Soc. Biol. **3**: 518, 1896.

50. Häuptli, O.: Zur Histologie der Poliomyelitis acuta und der Encephalitis epidemica (lethargica), Deutsche Ztschr. Nervenh. **71**:1, 1921.

51. Baker, A. B.; Matzke, H. A., and Brown, J. R.: Poliomyelitis: III. Bulbar Poliomyelitis: A Study of Medullary Function, Arch. Neurol. & Psychiat. **63**:257, 1950.

52. Matzke, H. A., and Baker, A. B.: Poliomyelitis: V. The Pons, A. M. A. Arch. Neurol. & Psychiat. **68**:1, 1952.

53. Matzke, H. A., and Baker, A. B.: Poliomyelitis: IV. A Study of the Midbrain, A. M. A. Arch. Neurol. & Psychiat. **65**:1, 1951.

54. Baker, A. B.; Cornwell, S., and Brown, I. A.: Poliomyelitis: VI. The Hypothalamus, A. M. A. Arch. Neurol. & Psychiat. **68**:16, 1952.

55. Baker, A. B.; Cornwell, S., and Tichy, F.: Poliomyelitis: IX. The Cerebral Hemispheres, A. M. A. Arch. Neurol. & Psychiat. **71**:435, 1954.

56. Baker, A. B., and Cornwell, S.: Poliomyelitis: X. The Cerebellum, A. M. A. Arch. Neurol. & Psychiat. **71**:455, 1954.

57. Hyland, H. H.; Gardiner, W. J.; Heal, F. C.; Oille, W. A., and Solandt, O. M.: Acute Anterior Poliomyelitis: A Review of 66 Adult Cases Which Occurred in the 1937 Ontario Epidemic, Canad. M. A. J. **39**:1, 1938.

58. Eyre-Brook, A. L.: Cerebrospinal Fluid in Acute Anterior Poliomyelitis, Brit. M. J. **1**:758, 1942.

59. Drury, J. C., and Sladden, A. F.: Cerebrospinal Fluid in Anterior Poliomyelitis, Brit. M. J. **2**:557, 1939.

Arteriosclerosis in the Elephant

Fatal Coronary Arteriosclerosis, with Report of an Autopsy

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Studies in this and other laboratories have shown that naturally occurring arteriosclerosis of the aorta and coronary vessels is not uncommon in several species of animals, namely, the dog,[1] cat,[2] pig,[3] and chicken.[4] The disease is usually severer in older animals, and, in the dog, involvement of the coronary arteries may lead to cardiac disability or death.[1] Because arteriosclerosis has not been described previously in the elephant, we are reporting a case of severe arteriosclerosis with fatal coronary insufficiency in this species.

The animal was a female Indian elephant that had lived in the San Francisco Zoological Garden since 1925 and was believed to be at least 47 years of age at the time of death. Its diet had consisted of oat hay supplemented by bread, lettuce, and carrots, and peanuts contributed by visitors to the zoo. Except for occasional bouts of constipation, the animal had never been ill and appeared healthy the night before death. On the morning of death, the elephant was found lying on its left side. The animal appeared conscious but was unable to rise. Examination by the zoo veterinarian, Dr. William E. Mottram, showed the animal to be in evident discomfort. Respirations were rapid, shallow, and labored. The abdomen was distended and tympanitic, and a tentative diagnosis of fecal impaction or bowel obstruction was made. The abdominal distention was not relieved by an enema. During the next two hours the animal's condition did not change; the elephant was restless and moved about the floor, although it remained on its left side. Respirations ceased suddenly.

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REPORT OF AUTOPSY

Gross Description

The autopsy was performed 19 hours after death of the elephant. The body was estimated to weigh 3.5 tons (3178 kg.). Rigor mortis was complete. No external abnormalities were noted except for a moderate amount of sanguineous fluid issuing from the distal end of the trunk. The thoracic and abdominal cavities were entered by reflecting the right thoracic and abdominal walls downward. The peritoneal cavity contained gas under considerable pressure. The serosal surfaces were smooth and glistening. Except for moderate distention of the colon with gas, the entire gastroenteric tract appeared normal and without evidence of obstruction or intussusception. The contents of the tract consisted of hay in varying degrees of digestion.

The pericardial cavity contained approximately 8 liters of sanguineous fluid. The heart was believed to be of normal size in relation to the size of the thoracic cavities and was estimated to weigh 30 to 40 lb. (13.6 to 18.1 kg.). The visceral pericardium was smooth and glistening, although considerable mottling and congestion over the surface of the left ventricle were observed. The entire heart had a soft, flabby consistency, and all chambers appeared moderately dilated. The myocardium of the left ventricular wall averaged 7 to 8 cm. in thickness and showed many poorly defined areas of pallor and congestion. No gross evidences of infarction and no myocardial scarring were observed. Visceral peri-

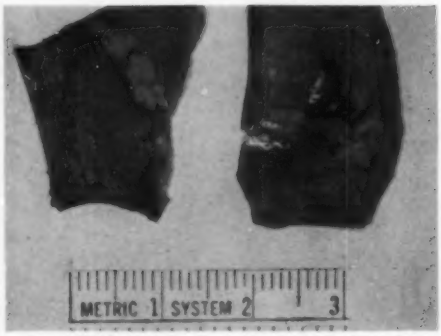

Fig. 1.—Segments of the anterior descending branch of the left coronary artery and of the pulmonary artery containing elevated and coalescing intimal fibrous plaques; × 1.4.

cardial and endocardial hemorrhages measuring up to 1 cm. in diameter were noted throughout the heart. On the cardiac surface they tended to be more numerous near the base and along the courses of the major coronary arteries. Beneath the endocardium these hemorrhages were more numerous in the left ventricle.

All cardiac valves appeared dilated. In the midportion of the superior surface of each aortic valvular cusp, irregular slightly elevated nodules or ridgelike plaques, 1 mm. in size, were observed. These were pale and fibrous and showed no gross evidences of lipid infiltration. Lying along the line of closure of the mitral valve and most numerous on the anterior leaflet were many 1 to 2 mm., gray-white, fibrous slightly elevated plaques or similar but elongated polypoid structures.

The major coronary arteries were patent and averaged 3 cm. in diameter in their proximal portions. A longitudinal row of slightly elevated, circumscribed, and coalescing white fibrous plaques, together measuring 2.5 cm. in length, was seen on the anterior wall of the upper portion of the anterior descending branch of the left coronary artery (Fig. 1). These plaques averaged 3 mm. each in diameter, had flat or slightly concave surfaces, and contained calcific deposits. The other major coronary arteries were free of arteriosclerotic lesions.

The pulmonary arteries were patent. In the midportion of the main pulmonary artery, on the anterior wall, was a group of slightly elevated ovoid and coalescing plaques, the largest measuring 0.5 cm. in diameter. These had gray surfaces and appeared fibrous without evident lipid infiltration (Fig. 1). The trachea and major bronchi displayed mucosal congestion and contained sanguineous fluid. Both pleural cavities were completely obliterated by fibrosis, and the lungs were firmly adherent to the thoracic walls and diaphragm. Both lungs were diffusely and severely congested, reddish-purple, and moderately firm. Little intra-alveolar fluid was present, and there was no consolidation.

The liver appeared to be of normal size. Its cut surface showed moderate central lobular congestion. The pancreas presented a uniform appearance in all portions, and its lobular pattern was distinct.

The elongated spleen appeared contracted and possessed a wrinkled, gray, intact capsule. The pulp was pale, fibrous, coarsely trabeculated, and depleted of blood.

The right adrenal gland was a flattened ovoid structure measuring 18×5×1 cm. Its cortex was pale yellowish-tan, and the smaller medullary segment was dark brown. The right kidney was estimated to be of normal size and weight. Since this organ was requested by the School of Veterinary Science for special studies, further examination was not made. The left adrenal gland and kidney could not be found even after prolonged search.

The bone marrow of the ribs was uniformly dark red and appeared to be normal. Nodular, hyper-

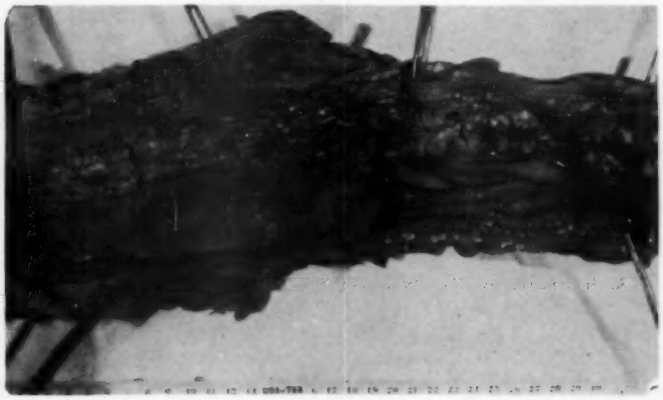

Fig. 2.—Lower thoracic aorta containing many flat intimal plaques, most numerous on the posterior wall. Note diffuse intimal wrinkling. Intimal staining is by hemoglobin; reduced about ½ from mag. × 0.6.

trophic, cartilaginous elevations were observed at the posterior margins of the articular cartilage of the upper end of the right humerus.

The uterus appeared atrophic and measured 11 cm. in length and 7 cm. in diameter. The uterine tubes were contracted and fibrotic. The ovaries averaged 7 cm. in diameter and were fibrotic and contracted with irregular serosal surfaces.

The arch of the aorta and the upper segment of the thoracic portion appeared entirely normal. The intima was smooth, glistening, and free of arteriosclerotic lesions. In the lower portion of the thoracic aorta, beginning at the level of the fourth intercostal arterial openings, there were many intimal plaques measuring between 0.5 and 1.0 cm. in their widest diameters. They were more numerous on the posterior wall than elsewhere and tended to be grouped about the intercostal arterial openings. The plaques became more numerous toward the lower portion of the thoracic aorta and upper portion of the abdominal aorta (Fig. 2). In these locations the plaques were widely distributed on the aortic intima and were present in greater numbers on the posterior wall. They were circumscribed and coalescing, and their outlines tended to be square or rectangular. Usually their widest diameters were parallel to the long axis of the aorta. These lesions, particularly the larger ones, were slightly elevated. Their surfaces were flat or slightly concave, pale yellowish-gray, and, in most cases, calcified. Between the plaques the intima was delicately wrinkled, as a rule, longitudinally. Intimal plaques were less numerous in the inferior segment of the abdominal aorta. A few identical intimal lesions were observed in the proximal segments of the common iliac arteries.

Microscopic Examination *

Heart.—The pericardial layer consisted of normal adipose tissue containing normal, small coronary arteries and veins. The myocardial fibers were of uniform size, and the majority of them displayed considerable transverse fragmentation, although the longitudinal and cross striations were still visible. Granular lipochrome pigment lay adjacent to the nuclei in most of the myocardial fibers. The left ventricular endocardial layer was mildly thickened and consisted of loosely arranged fibrils lying parallel to the endothelial surface. Near the endothelium the fibers were mainly reticulum, whereas in the deeper layer, collagenous fibrils were more numerous. Mucopolysaccharides and lipids were not demonstrable in this layer.

The aortic valvular cusps were covered by intact endothelium. The inferior portions of these cusps consisted of a uniformly thin layer of delicate fibrillary material containing numerous wavy, fine elastic fibers. Few fibrocytes were present. The superior layers of the cusps were composed of an irregular mass of hyaline connective tissue, which was relatively acellular and contained many irregular amorphous calcific deposits appearing to originate in the connective tissue cells and fibers. The thickened segments of the cusps contained abundant collagen and large amounts of acid mucopolysaccharide, localized, as a rule, about the calcific deposits. Moderately abundant lipid, appearing as intracellular droplets, was observed in the thickened segments of the aortic cusps and was generally deposited near the calcific structures. The lipid did not stain with Nile blue, nor was it refractile when examined with polarized light.

Projecting from the superior surface of the leaflets of the mitral valve were polypoid masses of pale, fibrillary, mucoid connective tissue containing a few compressed fibrocytes (Fig. 3). These polyps were covered by an intact layer of endothelium and consisted mainly of a dense meshwork of delicate fibrils that stained for mucopolysaccharide, although fine reticulum and collagenous fibers were present. These latter fibers were more numerous in the deeper portions, where a few delicate wavy elastic fibers were also observed. In the compact fibrous middle layer of the mitral valvular leaflets were several small arteries displaying concentric intimal thickening and extreme narrowing of their

* Multiple blocks of tissue from the cardiac valves, myocardium, major coronary arteries, pulmonary artery, and aorta were made, and contiguous frozen sections from each block were stained with Sudan IV and hematoxylin. An unstained frozen section was used for examination with polarized light. These blocks were then embedded in paraffin, and contiguous sections were treated with hematoxylin and eosin stain, Laidlaw's connective tissue stain, a combined Weigert-Van Gieson stain, and a colloidal iron-Prussian blue stain for mucopolysaccharides.

lumens. Their internal elastic membranes were fragmented and reduplicated, and the thickened intimas consisted chiefly of reticulum and collagenous fibers, although small amounts of mucopolysaccharide were also present. Few fibroblasts lay in the thickened intimas. No lipid was demonstrable in the mitral valvular tissues or in the small mitral arteries.

The majority of small coronary arterial branches in the left ventricular wall were diseased. The earliest lesions were characterized by segmental degeneration of the internal elastic membrane, which was fragmented, beaded, and usually displayed some degree of reduplication. Intimal thickening in these arteries was minimal or absent. Where it was widened, the intima consisted of a few delicate reticulum, collagenous, and mucopolysaccharide fibers, and a few fibroblasts. Lipid was absent in these earliest lesions.

Early evidences of disease were observed in a number of arteries averaging 0.1 mm. or less in diameter. The intima was concentrically or eccentrically thickened and was composed of plump fibroblasts and delicate intercellular reticulum, collagenous, and mucopolysaccharide fibers. The internal elastic membrane was fragmented, coarsened, and reduplicated. A few delicate elastic-tissue fibers in the intima were closely associated with intimal fibroblasts.

Severer lesions were observed in many medium-sized myocardial arteries which averaged 0.5 mm. in diameter. Some displayed only mild intimal thickening, consisting of delicate reticulum, collagenous, and mucopolysaccharide fibers. As a rule, the internal elastic membrane was fragmented and beaded, and often there was considerable reduplication, splitting, and fraying of the elastic tissue. Many coarse elastic fibers filled the intimas of some arteries (Fig. 4).

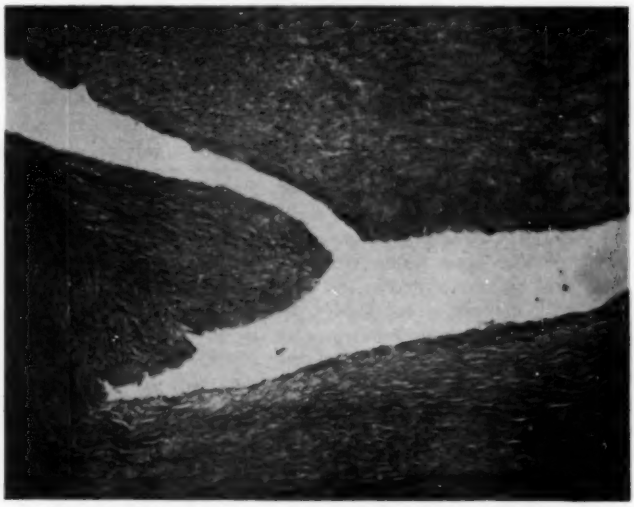

Fig. 3.—Segments of polypoid structures projecting from superior surface of mitral valvular leaflets. These lesions consist mainly of fibrillary material that stains for mucopolysaccharide. Colloidal iron-Prussian blue stain; × 100.

Many arteries had extremely thick intimal layers and tiny residual lumens (Figs. 5 and 6). The thickened intimas consisted mainly of coarse hyaline fibers, both reticulum and collagen. Relatively few fibroblasts with hyperchromatic nuclei were arranged irregularly between the fibers. Very small amounts of fibrillary material staining for mucopolysaccharide were demonstrable in the thickened intima (Fig. 6). No lipid was found in the walls of any of the small coronary arterial branches, even when severe intimal thickening was present.

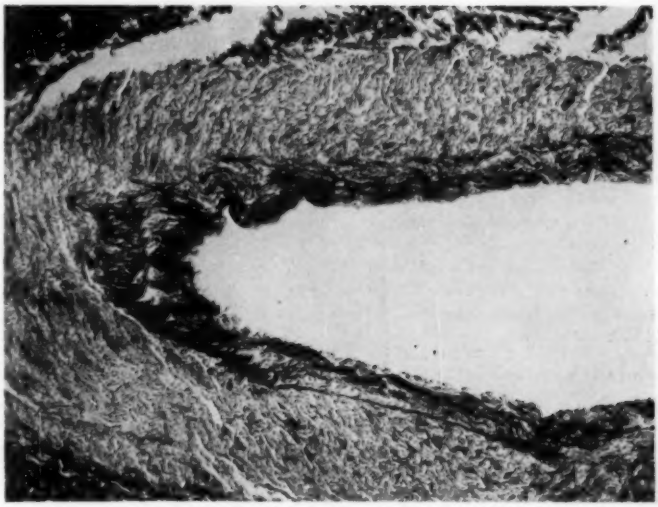

Fig. 4. — Coronary artery in left ventricular myocardium. The intima is thickened and contains many elastic fibrils. The internal elastic membrane is fragmented and reduplicated. Weigert-Van Gieson stain; × 200.

The media and adventitia of the small coronary arterial branches of the myocardium were entirely normal. No vascular lesions were observed in the right ventricular wall or in the interventricular septum.

Sections of the plaques in the anterior descending branch of the left coronary artery revealed considerable widening of the intima, which averaged 0.3 mm. in thickness. This was composed mainly of wavy collagenous fibers lying parallel to the endothelial surface (Fig. 7), and contained few elongated fibrocytes. Small groups of lymphocytes were present in the superficial layers of some portions of the thickened intima. The superficial intimal layer, as a rule, was distinctly fibrillar, whereas the deeper layers were more condensed and often hyalinized. An abundance of reticulum was present in all portions of the intima, and the fibers were coarser and more compact in the superficial layers. Delicate wavy fibrillary mucopolysaccharide substance was present in the superficial and in the deeper layers of the

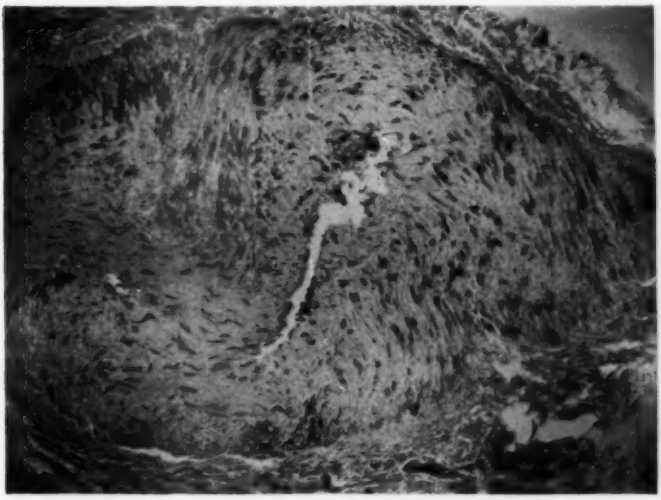

Fig. 5. — Coronary artery in left ventricular myocardium. Intimal thickening has caused subtotal occlusion of the lumen. Hematoxylin and eosin stain; × 100.

Fig. 6.—Coronary artery in left ventricular myocardium. The intima is hypertrophied and contains small groups of fibrils staining for mucopolysaccharide. Colloidal iron-Prussian blue stain; × 100.

intima, whereas this substance was absent in the midportion of the thickened intimal layer. Moderate numbers of delicate wavy elastic fibers also appeared in the intima, usually distributed near and surrounding the intimal fibroblasts.

The intima was poorly demarcated from the inner medial layer, which contained increased amounts of collagen and displayed considerable fragmentation of the inner medial elastic fibers. The internal elastic membrane was absent or was represented by a few fragmented, short, coarse, and reduplicated elastic segments. Many calcific deposits were observed in the intima. These originated as small granular deposits on the intimal collagenous fibers. Fusion of the deposits formed flattened elongated calcific plaques lying mainly in the deeper intimal layers. Even though extensively calcified, the fibrillar pattern was generally retained, although the previous intimal structure was obscured when the deposits became larger, more rounded, and amorphous (Fig. 8). Similar calcification also involved the superficial layer of the media, especially where the two layers

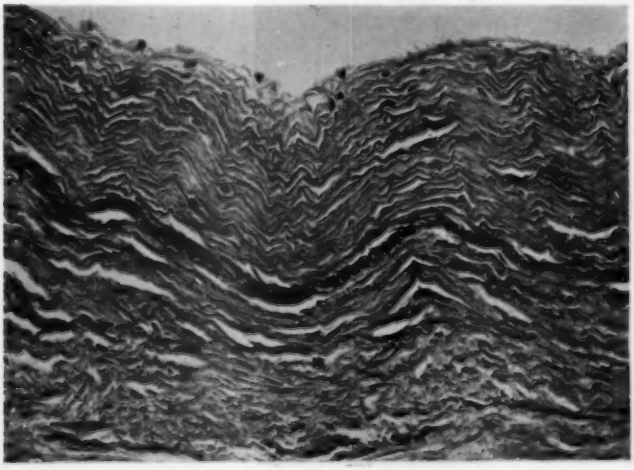

Fig. 7.—Intimal plaque in anterior descending branch of left coronary artery. Note thickening and irregularity of internal elastic membrane. Weigert - Van Gieson stain; × 200.

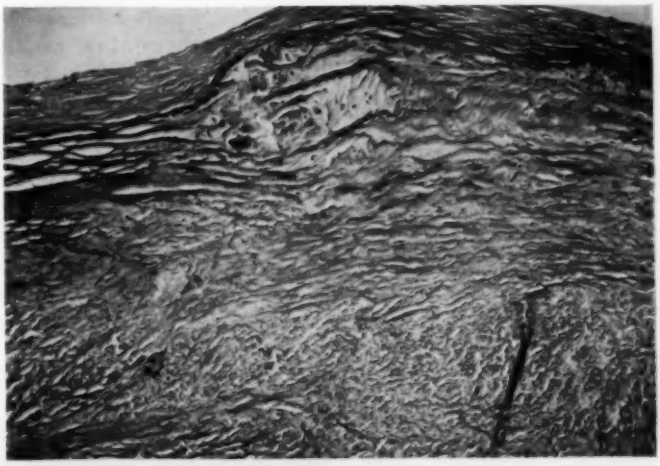

Fig. 8.—Intimal plaque in anterior descending branch of left coronary artery showing intimal and medial fibrosis and nodular calcification. Weigert-Van Gieson stain; × 100.

were so intimately fused. Abundant finely divided lipid droplets were demonstrable in the thickened intimal layer and were numerous in the deeper intima, as well as in the superficial media. The lipid was mainly intracellular and was most abundant in intimal cells in and adjacent to those undergoing calcification. It did not stain with Nile blue, nor was it visible with polarized light. The outer medial and adventitial layers appeared normal and contained normal amounts of acid mucopolysaccharide.

Similar lesions were observed in the right coronary artery. However, intimal thickening was less pronounced, calcification was minimal, and only small amounts of lipid were found in the deeper portions of the intimal layer. The circumflex branch of the left coronary artery displayed intimal lesions almost comparable in extent with those in the anterior descending branch of the left coronary artery. Calcification was minimal. The deeper layers of the thickened intima consisted mainly of collagen, whereas the superficial layers contained abundant fibrillary mucopolysaccharide. Lipid infiltration in the deep intima was slight and was localized near the small calcific deposits.

Pulmonary Artery.—Moderate thickening of the intima of the main pulmonary artery

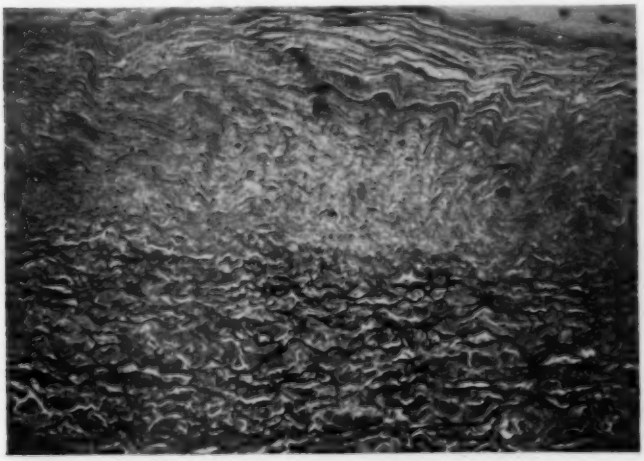

Fig. 9. — Pulmonary artery displaying intimal widening with wavy collagenous fibers. Weigert-Van Gieson stain; × 100.

resulted in plaque formation (Fig. 9). The plaque was formed by fibrillary substance arranged parallel to endothelial surface. Few fibrocytes were present. Abundant delicate reticulum, collagenous, and mucopolysaccharide fibers were present in the intimal layer, although collagen was most plentiful superficially. A few delicate wavy elastic fibers in the deeper layers were originating from the superficial segments of the medial elastic tissue. A few scattered, finely divided intracellular lipid droplets were present in the intimal layer. This substance did not stain with Nile blue or appear under polarized light. Small segments of the medial elastic tissue had been separated or replaced by deposits of collagenous and mucopolysaccharide substances. The adventitia was not altered.

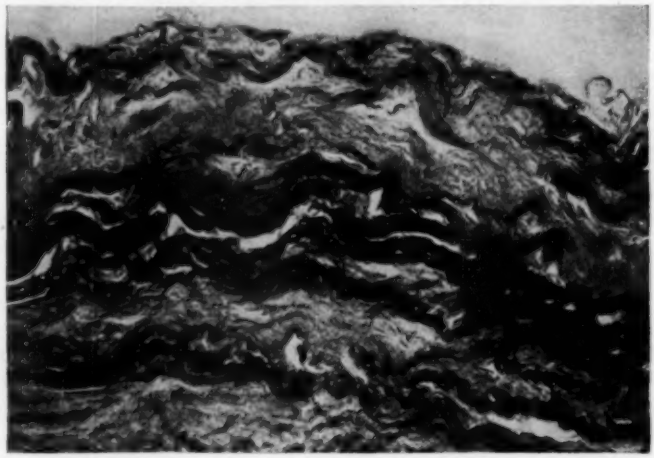

Fig. 10.—Inner segment of upper thoracic aorta with coarsening and condensation of inner medial elastic fibers. Weigert-Van Gieson stain: × 500.

Aorta.—Thoracic Arch: The intima at this level was mildly thickened and consisted of delicate loose reticulum, collagenous, and mucopolysaccharide fibers. The superficial elastic layers of the media were fragmented, and some fibers extended into the loosened intima. Fine intracellular and extracellular lipid droplets in small numbers were observed in the intimal layer. This substance did not stain with Nile blue and was not visible with polarized light. The muscular and elastic pattern of the media appeared normal, and small amounts of mucopolysaccharide surrounded the medial elastic fibers. Treatment of duplicate sections with testicular hyaluronidase only partially removed the intimal and medial mucopolysaccharide.† In the midportion of the media were several large nerves, usually containing normal medium-sized arteries. The adventitia of the thoracic arch was not altered.

Upper Thoracic Aorta: At this level there was no intimal thickening. Beneath the endothelium the elastic fibrils were condensed considerably (Fig. 10). In the inner third of the media, elastic fibers were disrupted and fragmented, and were often surrounded by pools of mucopolysaccharide or replaced by irregular segments of collagenous connective tissue (Fig. 11). Increased amounts of fibrillary mucopolysaccharide substance and scattered, fine lipid droplets were present in the superficial layer of the media beneath the endothelium. Incubation with testicular hyaluronidase completely removed the mucoid substance from the intima and inner media, whereas mucopolysaccharide of the outer media was only partially destroyed.

Abdominal Aorta: Disease was most pronounced in the lower thoracic and upper

† The hyaluronidase was supplied by The Wyeth Institute for Medical Research.

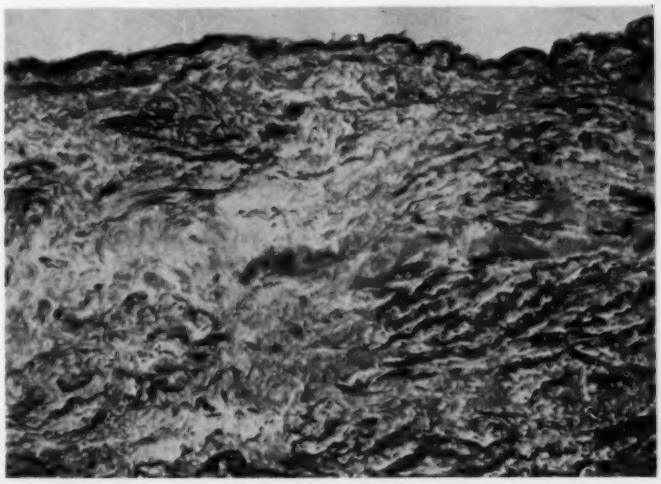

Fig. 11.—Upper thoracic aorta. The elastic fibrils are degenerating and fragmented, and many are replaced by collagen. Weigert - Van Gieson stain; × 100.

abdominal segments. The intima was altered, but degenerative changes were severest in the inner third of the media. The intima was only mildly or moderately thickened and consisted in part of fibrillary material and in part of hyaline connective tissue containing few fibrocytes (Fig. 12). Most of the fibrillary substance was mucopolysaccharide, and a few fine reticulum and a few deeply placed collagenous fibers were also present. The internal elastic membranes were coarsened, beaded, fragmented, and reduplicated, and large segments had completely disappeared. The intimal thickening was greater at the site where the internal elastic membrane was absent or where medial changes were most pronounced. The few delicate, wavy elastic fibers observed in the deeper portions of the thickened intima seemed to be derived from the intimal connective-tissue cells rather than from the preexisting internal elastic membrane.

The inner one-half to two-thirds of the media displayed hyaline fusing of the smooth muscle cells and extensive loss of elastic fibers, which appeared as short fragmented

Fig. 12. — Abdominal aortic plaque. Moderate intimal thickening is present. The smaller medial calcific deposits are applied to single smooth muscle cells. Hematoxylin and eosin stain; × 200.

segments. Extensive deposits of coarse fibrillary mucopolysaccharide were irregularly interspersed with heavy collagenous fibrils in this inner medial segment. Few smooth muscle nuclei remained. Treatment of these abdominal aortic sections with testicular hyaluronidase removed most of the intimal and medial mucopolysaccharides, leaving small amounts adjacent to the medial elastic fibers and calcific deposits.

Extensive calcification had occurred in the inner one-third to one-half of the media. The calcific deposits originated as tiny calcific granules, appearing first on individual smooth muscle cells or their nuclei. Thus the smaller calcific masses were fusiform, corresponding to the shape of these cells. As the calcific deposits enlarged, fusion into flat medial plaques occurred, although the original muscular fiber pattern persisted, as a rule (Fig. 12). Some calcific masses were larger, more rounded, and amorphous. These usually elevated the intima (Fig. 13). The combined disease of the intima and media with loss of the demarcating internal elastic membrane fused the two layers intimately.

Fine lipid droplets were observed in the deeper portions of the thickened intima and in the inner media. Generally the lipid was congregated about and near the calcific deposits. This substance did not stain with Nile blue or appear with polarized light.

Medial mucopolysaccharide was particularly abundant in and about the calcific deposits in the inner third of this layer. The outer third of the media had a uniform muscular and elastic pattern, and small amounts of mucopolysaccharide enveloped the individual elastic fibers. A wide adventitial layer consisted of coarse collagenous bundles interspersed with abundant adventitial elastic tissue.

Iliac Arteries.—Lesions in these vessels were identical with but less severe than those observed in the abdominal aorta. Calcification of the inner media was moderately extensive.

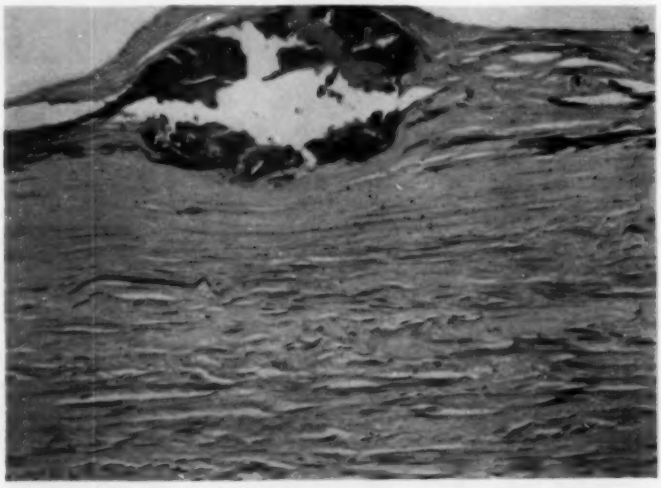

Fig. 13. — Abdominal aortic plaque. The nodular calcific deposit lies in both the intimal and medial layers. Note hyaline fusing of inner segment of the medial muscular layer. Hematoxylin and eosin stain; × 100.

Lungs.—Pronounced vascular congestion was observed in all portions of both lungs. All alveoli were filled with serous fluid, and many with intact red blood cells. Considerable interstitial hemorrhage had also occurred. Except for the presence of blood in the lumens, the bronchi and bronchioles were not abnormal. No inflammatory reaction was observed. One pulmonary section contained a single rounded mass of hyaline connective tissue surrounded by a few infiltrating lymphocytes. This lesion resembled a healed tubercle.

Liver.—The hepatic lobules had a normal structure. The majority of parenchymal cells were normal, although the central lobular cells, as a rule, contained granular lipochrome pigment. The portal areas, the sinusoids, and the central venous tributaries were not unusual.

Kidney.—The majority of the glomeruli were two or three times the size of those of human beings or other mammals. Glomerular vascularity was decreased, and few of the capillaries contained blood. The glomerular basement membranes were frayed and reduplicated. Each glomerulus contained several greatly enlarged epithelial cells with oval nuclei, abundant particulate chromatin, and prominent nucleoli. The cytoplasm was relatively scanty. A few epithelial nuclei were large, dense, and hyperchromatic. The convoluted tubular epithelial cells were swollen as a result of postmortem degeneration. The interstitial connective tissue was loosened and fibrillary. No arterial lesions were observed in the renal parenchyma.

COMMENT

Few autopsy reports on elephants have been made. Benedict [5] collected 19 such reports and summarized the original observations of Aristotle and Galen. One of these reports was by Fox, who later cited reports of four autopsies which he had performed on elephants.[6] Galen (cited by Benedict [5]) described a sclerotic cardiac condition as "a bone of the heart." Fox (cited by Benedict [5]) in 1908 observed areas of thickening surrounded by pale fibrous zones about the opening of the left coronary artery. Myocardial fibrosis associated with infiltration of plasma cells and a few lymphocytes was observed in the same animal. The aorta was smooth, pale yellow, and normally resilient. In his descriptions of arteriosclerosis in a variety of animals, Fox specifically noted the absence of arteriosclerosis in the elephants which he examined.[6]

Arterial disease in the elephant described in this paper was similar in many respects to that observed in other animals. In the small coronary and mitral valvular arteries, the fragmentation and degeneration of the internal elastic membrane followed by deposition of collagenous and reticulum fibers were identical with that observed in the muscular arteries of birds,[4] dogs,[1] cats,[2] and human beings.[7] The intimal lesions of the small coronary arteries of the elephant, although subtotally occluding many vascular lumens, were devoid of lipid. It should be noted that similar lesions without infiltrating lipid in the coronary arteries may cause cardiac disability or sudden death in old dogs.[1]

Similar intimal disease leading to the formation of intimal plaques was observed in the major coronary arteries, in the pulmonary artery, and in the aorta. Degeneration of the internal elastic membrane preceded the intimal deposition of mucopolysaccharide and connective-tissue fibers.

Medial disease associated with calcification was a prominent feature of the sclerotic process in the aorta and, to a slighter degree, in the major coronary arteries. This medial process was also regarded as resulting from medial elastic degeneration leading to medial accumulations of mucopolysaccharide and collagen. Although little lipid was present in the intimal and medial lesions of these large muscular and elastic arteries, lipid deposition appeared to be clearly a secondary process and not concerned in the early development of the arterial disease. Cholesterol was not demonstrated in the lesions. Prominent calcification of the media and, to a slighter extent, of the intima seemed secondary to the arterial elastic and muscular degeneration rather than to lipid infiltration. This calcific medial disease resembled aortic medial sclerosis in the human being [8] and the medial calcific lesions commonly observed in old cows.[6]

The course of the illness and the autopsy findings indicate that the immediate cause of death was acute myocardial failure, causing terminal pulmonary congestion, edema and hemorrhage, and hemorrhagic pericardial effusion. The cardiac failure appeared to have resulted from diminished coronary blood

flow due to severe arteriosclerosis of many small coronary arterial branches. Sudden death from acute myocardial failure resulting from similar disease of small coronary arteries has also been observed in the human being, in the absence of a significant narrowing of the major coronary arteries.‡

SUMMARY

The clinical and autopsy findings in a 47-year-old female Indian elephant are described.

Severe aortic arteriosclerosis was observed and was characterized by intimal thickening and extensive medial degeneration and calcification. Similar disease involved the major coronary and pulmonary arteries.

The cause of death was acute cardiac failure, secondary to pronounced arteriosclerosis of many small coronary arterial branches. No lipid or cholesterol was present in these small diseased coronary arteries.

Mr. Carey Baldwin, Director of the San Francisco Zoological Garden, made this animal available for study, and Mr. Hal Strong of the Veterans Administration Hospital, Oakland, Calif., made the photographs.

‡ Lindsay, S.: Unpublished data.

REFERENCES

1. Lindsay, S.; Chaikoff, I. L., and Gilmore, J. W.: Arteriosclerosis in the Dog: I. Spontaneous Lesions in the Aorta and the Coronary Arteries, A. M. A. Arch. Path. **53**:281-300, 1952.

2. Lindsay, S., and Chaikoff, I. L.: Arteriosclerosis in the Cat: Naturally Occurring Lesions in the Aorta and the Coronary Arteries, A. M. A. Arch. Path. **60**:29-38, 1955.

3. Gottlieb, H., and Lalich, J. J.: Occurrence of Arteriosclerosis in the Aorta of Swine, Am. J. Path. **30**:851-855, 1954.

4. Lindsay, S.; Nichols, C. W., Jr., and Chaikoff, I. L.: Aortic Lesions Induced in the Bird by Diethylstilbestrol Injections and Cholesterol Feeding: A Study of Their Development and Regression, A. M. A. Arch. Path. **59**:173-184, 1955.

5. Benedict, F. G.: Physiology of the Elephant, Washington, D. C., Carnegie Institution of Washington, 1936.

6. Fox, H., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, Ch. 6, pp. 153-193.

7. Moon, H. D., and Rinehart, J. F.: Histogenesis of Coronary Arteriosclerosis, Circulation **6**:481-488, 1952.

8. Blumenthal, H. T.; Lansing, A. I., and Wheeler, P. A.: Calcification of the Media of the Human Aorta and Its Relation to Intimal Arteriosclerosis, Ageing and Disease, Am. J. Path. **20**: 665-687, 1944.

News and Comment

PERSONAL

Leslie Dana Award to Mrs. Helenor Wilder Foerster.—Mrs. Helenor Wilder Foerster, former chief of the ophthalmic pathology branch of the Armed Forces Institute of Pathology, has been awarded the Leslie Dana Gold Medal for 1955. This medal is awarded annually by the St. Louis Society for the Blind to a winner selected by the National Society for the Prevention of Blindness. Mrs. Foerster is still active as a consultant to the Armed Forces Institute of Pathology.

Appointments.—Dr. Stanley H. Durlacher, formerly of the Orleans parish coroner's office, has become medical examiner for Dade County, Fla.

Dr. John B. Alsever has been appointed full-time medical director of Southwest Blood Banks, Inc., with headquarters in Phoenix, Ariz. Dr. Alsever was formerly instructor in pathology at Syracuse University College of Medicine.

Dr. Rudolph J. Muelling Jr., of New Orleans, has recently been appointed pathologist to the Orleans parish coroner's office.

Some Observations on Calcifying Cartilage Matrix

D. JOE FREEMAN, M.D., Madison, Wis.

INTRODUCTION

The mechanism whereby mineral salts are deposited in cartilage matrix is a complex one probably involving the action of alkaline phosphatase, glycolysis, alterations in the ground substance, vitamins, and hormones; in addition, there may well be unknown factors such as enzymes, pH changes, etc., which are intimately tied up in this process. The role of vitamins and hormones seems to be more one of regulation than one of causation.

The function of alkaline phosphatase in calcification has been intensely investigated, but nevertheless remains obscure. Robison's [1] original hypothesis that the enzyme raises the concentration of phosphate to such a degree that precipitation occurs from the locally supersaturated medium is attractive but unproved. More recent evidence suggests that the hexosephosphoric esters formed during the degradation of glycogen may well serve as substrates for alkaline phosphatase according to this theory.* In the last analysis then, this concept explains calcification upon the basis of "mass law considerations of solubility equilibria." [4] Robison himself, however, discusses a so-called "second mechanism" which must be acting to explain the process more completely.

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Resident in Medicine, University Hospitals, Madison, Wis.

* References 2 through 5.

† References 6 through 10.

Other investigators † have suggested that alterations in the cartilage matrix or ground substance, imparting on it the property of "calcifiability," may well be this "second mechanism" (or the "local factor"). The organic material of cartilage matrix consists of the acid mucopolysaccharide, chondroitin sulfate, and collagen protein. Whether or not alterations in the protein and/or the chondroitin sulfate are the fundamental ones concerned with this property of "calcifiability" is undetermined. In decalcified sections there is a marked increase in the intensity of the metachromatic staining of the matrix in the regions of previous calcification.[7] This is strong suggestive evidence that some alteration in chondroitin sulfate, presumably a depolymerization, has occurred coincident with the onset of calcification. It has also been demonstrated that there is a progressive resistance to the action of hyaluronidase of maturer cartilage matrix (not including calcified matrix). It has been shown in this laboratory [11] and by Fawns [12] that hyaluronidase will attack calcified cartilage matrix with little success unless it has been decalcified. Dziewiatkowski [13] and Bélanger [14] have observed, in autoradiographs of young rats fed radiosulfate and killed at various intervals, the presence of the isotope in all cartilage areas. The maximum concentration is in the zone of young proliferating hypertrophic cartilage cells and, probably to a slighter extent, in the region of the older hypertrophic cells. The isotope appears first in the cells, then, in the cell and matrix, and, after six days, it is found only in the matrix. These authors suggest that a more active formation of chondroitin sulfate occurs in this hypertrophic area.

This hypothesis, then, states that chondroitin sulfate is somehow altered or formed so that there is an increased density of reactive acid groups which make the cartilage calcifiable by binding bases.

On the other hand, some investigators ‡ believe that the protein of the matrix may play the significant role. Alkaline phosphatase is an important component of cells engaged in the rapid synthesis of proteins in other tissues.[17] Studies of decalcified bone transplants have suggested that alkaline phosphatase is closely linked with a cell (the so-called "matrix cell") which is concerned with the formation of the fibrous-like, "noncalcifiable" matrix structure of adult bone.[18] Studies of bone with use of the electron microscope have demonstrated the orientation of bone mineral crystals at the sites of "bridges" between the collagen fibers.[10] Whether or not these "bridges" represent mucopolysaccharide protein molecules or protein molecules of the collagen system could not be determined. In cartilage matrix at least definite changes in the chondroitin sulfate have been demonstrated. Whether or not associated changes occur in the collagen protein cannot be stated.

Still other investigators explain calcification largely as a matter of surface chemistry.§ Their work suggests that alkaline phosphatase may be acting as the trigger mechanism for calcification by removing an agent (presumably adsorbed ester phosphate) which hitherto had prevented the precipitation of calcium phosphate salts from the matrix fluid. Thus, they postulate that calcification occurs by such phenomena as adsorption, recrystallization, and lattice formation.

Perhaps too little attention has been directed toward the energy requirements of calcifying cartilage and bone. At present most investigators believe that the energy released by the cycle of phosphorylative glycogenolysis is largely an inconsequential result of building up local concentrations of phosphate esters or ions. Harris [2] has demonstrated the rapid decrease in the concentration of intracellular glycogen in the zone of hypertrophic cartilage. The compound is present in all other cartilage areas, but apparently to a somewhat greater degree in the zone of young hypertrophic cells. Cobb has recently demonstrated the maximum concentration of the enzyme phosphorylase in this same area.[22] The possibility that glycogenolysis occurs in the hypertrophic cartilage cell area for the primary purpose of producing energy for matrix alterations and/or molecular productions cannot be ignored.

This study is concerned mainly with cartilage matrix changes during calcification. By the use of various histochemical procedures, hyaluronidase, and the technique of qualitative historadiography, some observations have been made which lend support to the concept of matrix alteration being the essential resulting in the property of "calcifiability" in cartilage.

MATERIALS AND METHODS

These studies were performed on 18-day-old rat fetuses.[11] All tissues (except those used for glycogen studies) were fixed in cold 85% ethyl alcohol and subsequently embedded in paraffin. The tissues used for the glycogen studies were fixed in 95% alcohol. Serial sections, 6μ in thickness, of the blocked cartilage bone tissues were used in almost all instances. Frequently, several procedures were carried out on the identical section.

Histochemical Techniques.—The following histochemical techniques were used: (1) hematoxylin and eosin stains; (2) von Kossa's technique for the demonstration of calcium; (3) Best's carmine stain to demonstrate glycogen; (4) Gomori's revised technique to demonstrate sites of alkaline phosphatase activity, performed on control and decalcified sections ‖; (5) toluidine-blue stain (0.5%, Grübler's), used to demonstrate metachromasia in controls, decalcified sections, and sections treated with hyaluronidase.

The decalcifying technique of Greep and co-workers,[23] with use of sodium citrate and formic acid, was used in all cases. Incubation periods of 10 minutes were found adequate to completely de-

‡ References 8, 15, and 16.

§ References 20 and 21.

‖ Calcium replacement by silver (von Kossa method) was substituted for the cobalt sulfide method recommended by Gomori.

mineralize these six microsections. The efficacy of the procedure was substantiated by control von Kossa stains and by microincineration. Subsequently, two washes in distilled water were employed to remove extraneous materials. This step was particularly important when historadiography was to be performed in order to prevent distorted historadiograms.

Control and decalcified sections were incubated at 37.5 C for 5, 15, 20, 25, 30, 60, and 90 minutes in solutions of hyaluronidase (Alidase) containing 500 viscosity units per cubic centimeter. Prior to incubation all sections were deparaffinized, rinsed, and air-dried. Similar sections were decalcified after incubation with hyaluronidase in order to demonstrate the metachromasia of calcified spicules in the bone marrow and osteoid.

Historadiograms were done on air-dried sections. The technique of historadiography is adequately described elsewhere.[24] Some historadiograms were performed on the identical section prior to and after incubation with hyaluronidase for 15 minutes. Only qualitative optical density has been estimated in these studies. With quantitative historadiograms[25] actual density (mass) can be measured. Using this technique, Clemmons¶ has recently done some studies on the bone of newborn rats, which will be mentioned below.

RESULTS

Follis[26] has subdivided cartilage into four areas: A is the area of isolated immature cartilage cells; B, that of paired immature cells; C, that where these paired cells have lined up into parallel longitudinal columns, and D, the hypertrophic area (zone of provisional calcification) where the cells enlarge, begin to lose their identity, and eventually disappear. For convenience of description, these divisions are used in this study; in addition, area D has been further subdivided into zones D-1 and D-2 for reasons which are discussed below. Zone D-1 is the relatively small area of hypertrophic cartilage in which the cartilage cells are beginning to enlarge; zone D-2 comprises the larger area of more truly hypertrophic cells which gradually lose their identity as they approach the bone marrow.

1. *Calcium.*—Calcium is absent from zone D-1 and is present in increasing amounts in zone D-2, in the cartilage spicules of the bone marrow, and in osteoid.

2. *Glycogen.*—The distribution of glycogen in all areas of cartilage, with a rapid decrease in zone D-2, is as described by Harris and Cobb. There is no readily apparent increase in glycogen concentration in zone D-1. Glycogen is also present in large amounts in the osteoblastic elements of the bone marrow and periosteum.

3. *Alkaline Phosphatase.*—Alkaline phosphatase is distributed maximally in zone D-1 and, in decalcified sections, its concentration

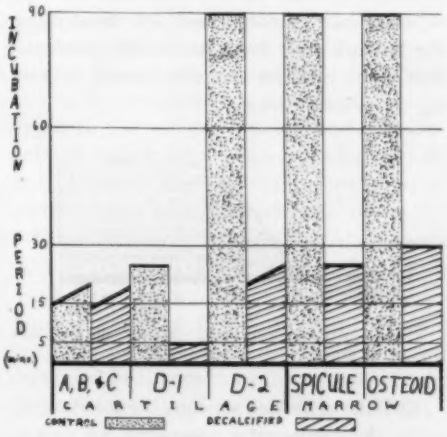

Fig. 1.—Comparative effect of hyaluronidase on cartilage ground substance in control and decalcified sections.

gradually decreases in the matrix of zone D-2. Alkaline phosphatase is present in trace amounts intracellularly in the other cartilage areas, A, B, and C. In addition, large quantities are found in the bone marrow and periosteum.

4. *Metachromatic Material.* — Metachromatic material (presumably chondroitin sulfate) was found as described by others. This staining reaction is somewhat more intense in zone D-1, but most intense in zone D-2 and cartilage spicules of the bone marrow in decalcified sections. Osteoid, when decalcified, also gives a faint reaction.

¶ Clemmons, J. J.: Personal communication to the author.

5. *Hyaluronidase Studies* (Fig. 1).—(*A*) In control sections the intensity of the metachromasia in cartilage matrix was unchanged after five minutes' incubation; that of noncartilagenous structures had disappeared. After incubation periods of from 15 to 25 minutes the metachromatic reaction rapidly disappeared, area A being most susceptible to the enzyme's action while traces of metachromasia remained in zone D-1 after 25-minute periods. There was a marked resistance of zone D-2 matrix to the enzyme, metachromasia being present following 90 minutes' incubation. The metachromasia of cartilage spicules in the bone marrow and osteoid was demonstrated by decalcifying the sections after incubation with hyaluronidase. The reaction was still present following 90-minute periods.

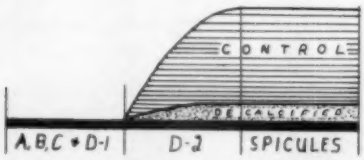

Fig. 2.—Historadiographic density of cartilage matrix in control and decalcified sections.

(*B*) In decalcified sections the matrix generally demonstrated a somewhat less resistance to the action of the enzyme with shorter incubation periods; however, two striking findings were obtained. Zone D-1 demonstrated essentially no metachromasia after 15-minute incubation periods, whereas traces of metachromasia remained in all other cartilage areas including A, B, and C. In addition, there was a progressive increase in the resistance of zone D-2 matrix to the enzyme's action. Metachromatic material was still present in this region, as well as cartilage spicules of the bone marrow, after 25-minute incubation periods. After 20 minutes' incubation with hyaluronidase no metachromasia remained in areas A, B, and C. Traces of metachromasia may or may not be present in osteoid tissue after 30 minutes' incubation with the enzyme. Ninety-minute incubation periods result in complete loss of metachromasia from all areas.

6. *Historadiographic Studies* (Fig. 2).—(*A*) Historadiograms taken on the identical section prior to and after decalcification demonstrated a progressive increase in the density of zone D-2 matrix; D-2 matrix had approximately the same density as cartilage spicules in the bone marrow and osteoid. More accurate measurement with quantitative historadiograms of newborn rat bone have confirmed this finding and demonstrated that osteoid has a somewhat greater density than cartilage spicules.#

(*B*) Historadiograms taken of decalcified sections prior to and after incubation with hyaluronidase for 15 minutes demonstrated no significant optical differences in the various cartilage areas. However no attempt has been made to confirm this observation by more accurate measurement with quantitative techniques. Control toluidine-blue staining of these identical sections demonstrated the same pattern of metachromasia as described above for this 15-minute incubation period.

COMMENT

In summarizing these observations certain differences in the areas and zones of cartilage are seen (Table). These observations suggest that there is indeed an alteration in cartilage matrix conferring on it the property of "calcifiability." This alteration begins, and probably is at its maximum, in zone D-1 but continues in the adjacent zone D-2 at gradually decreasing levels. Concomitantly, calcification per se begins in early zone D-2 and reaches its maximum in the areas adjacent to the bone marrow (Fig. 3). The alterations which occur are probably of at least four (or more?) varieties:

1. The removal of the loose bond of "adsorbed" calcium and ester phosphate with the ground substance.

2. A depolymerization of chondroitin sulfate. This change is probably maximum in

Clemmons, J. J.: Personal communication to the author.

Differences in the Various Areas and Zones of Calcifying Cartilage

	A, B, and C	D-1	D-2	Spicules (Marrow)
Calcium	None	None	Gradual increase	Resorption
Glycogen	Present	Present	Gradual decrease	.....
Alkaline phosphatase	Intracellular traces	Maximum	Gradual decrease	Trace
Phosphorylase	Present	Maximum	Gradual decrease	?
S^{35} uptake	Trace	Maximum	Gradual decrease	?
Metachromasia				
Control	Moderate, gradual increase		Gradual decrease to slight	
Decalcified	Moderate, gradual increase		Sharp increase to maximum	
Hyaluronidase resistance				
Control	Moderate, gradual increase		Very sharp increase to maximum	
Decalcified	Moderate (slightly less)	Minimum	Gradual increase to maximum	
Matrix density				
Control	Constant	Constant	Sharp increase	
Decalcified	No change	No change	More gradual increase	

zone D-1 and is suggested not only by the increased metachromasia of this zone but also by its unusual susceptibility to the action of hyaluronidase in decalcified sections.

Why should decalcification make this zone so susceptible to the action of the enzyme? Calcium and phosphate are bound to the matrix in all cartilage areas and probably in gradually increasing amounts as the cartilage matures from area A to zone D-1. This may occur because of a corresponding increase in the density of chondroitin sulfate in these areas (a possible explanation for the increased resistance to the action of hyaluronidase manifest in control sections in zone D-1). Decalcification must remove these loosely bound materials, freeing more surface against which hyaluronidase can act. Perhaps this is the reason decalcified sections as a whole are somewhat less resistant to the enzyme, with the least resistance being in

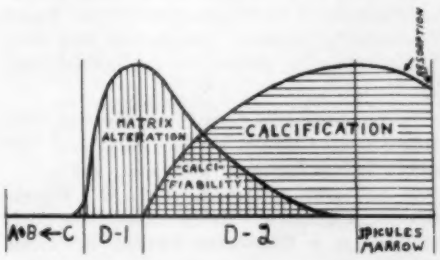

Fig. 3.—Graphic representation of the sequential events occurring in calcifying cartilage.

depolymerized zone D-1.* In addition to depolymerization, another possible explanation for the unusual susceptibility of this zone D-1 to hyaluronidase in decalcified sections might be the fact that shorter chained molecules of chondroitin sulfate are being formed in this area. Other factors, as yet undetermined, might also be important.

3. An increased formation of chondroitin sulfate. The radiosulfate studies of Bélanger leave little doubt of this. Additional support of this idea is the increased staining characteristics of these zones as well as the increased resistance to hyaluronidase and the increased density of decalcified zone D-2.

4. A fourth possible alteration is the coupling of chondroitin sulfate molecules to themselves (polymerization) and to matrix collagen protein. These changes probably begin and reach their maximum in zone D-2. The crucial change occurring before any calcium deposition develops may be a coupling of chondroitin sulfate to the protein matrix

* That the presence of a simple in vitro precipitate of calcium phosphate renders an area less susceptible to hyaluronidase has been demonstrated in this laboratory. Artificial calcification using the technique of Gomori to demonstrate alkaline phosphatase maintains the metachromasia of zone D-1 decalcified sections even after 90 minutes' incubation with the enzyme. Incidentally, the metachromatic character of this zone is also blocked by the presence of this precipitate, metachromasia being demonstrated by subsequent decalcification. Certain observations suggest that this type of calcification is definitely not physiological even in zone D-2.[11]

forming "bridges" analogous to those described by Robinson and co-workers in bone. Initial mineral deposition then occurs on these basic structural units. In addition to an increase in their formation, the coupling of these groups to matrix protein may also explain the increased resistance which zone D-2 matrix demonstrates to hyaluronidase in decalcified sections.

Accordingly, the sum total of these alterations (and others?) impart on the matrix the property of "calcifiability" and mineral deposition begins (Fig. 3). Further calcification might well be largely on the basis of surface chemistry (i. e., crystal adsorption, recrystallization, and lattice formation).

In addition to the performance of osmotic work, phosphorylative glycogenolysis probably has as its chief function the liberation of sufficient energy to drive these molecular productions and rearrangements to completion. Its prime function is most likely not the liberation of phosphates for combination with calcium; however, this may be a secondary gain.

SUMMARY

Cartilage bone sections of 18-day-old rat fetuses have been subjected to various histochemical techniques, incubated with hyaluronidase, and studied historadiographically. Comparison between controls and decalcified sections has resulted in certain observations which lend support to the concept of matrix alteration being the essential resulting in the property of "calcifiability" in cartilage.

This paper is based on studies done in 1950 and 1951 while I was a Research Assistant under the supervision of D. Murray Angevine, M.D., Chairman of the Department of Pathology, University of Wisconsin Medical School. Dr. Angevine and J. J. Clemmons, Ph.D., gave helpful suggestions and criticisms. This study was supported by the Wisconsin Alumni Research Foundation and the Atomic Energy Commission AT (11-1)-64 Project 8.

REFERENCES

1. Robison, R., and Rosenheim, A. H.: Calcification of Hypertrophic Cartilage in Vitro, Biochem. J. **28**:684, 1934.

2. Harris, H. A.: Glycogen in Cartilage, Nature London **130**:996, 1932.

3. Glock, G. E.: Glycogen and Calcification, J. Physiol. **98**:1, 1940.

4. Gutman, A. B., and Yu, T. F.: A Concept of the Role of Enzymes in Endochondral Calcification, in Transactions of the Second Conference on Metabolic Interrelations, New York, Josiah Macy, Jr. Foundation Publications, 1950, p. 167.

5. Conference discussion, in Transactions of the Fifth Conference on Metabolic Interrelations: With Special Reference to Calcium, New York, Josiah Macy, Jr. Foundation Publications, 1953, p. 114.

6. Sobel, A. E.: Local Factor in Calcification, in Transactions of the Second Conference on Metabolic Interrelations, New York, Josiah Macy, Jr. Foundation Publications, 1950, p. 113.

7. Howard, J. E.: Some Current Concepts on the Mechanism of Calcification, J. Bone & Joint Surg. **33-A**:801, 1951.

8. Roche, J.: La Phosphatase des os et le mécanisme général de l'ossification, Experientia **2**: 325, 1946.

9. McLean, F. C.; Lipton, M. A.; Bloom, W., and Barron, E. S. G.: Biological Factors in Calcification in Bone, in Transactions of the 14th Conference on Metabolic Aspects of Convalescence, New York, Josiah Macy, Jr. Foundation Publications, p. 9.

10. Sobel, A. E.: Studies on the "Local Factor" of Calcification, in Transactions of the Fourth Conference on Metabolic Interrelations, New York, Josiah Macy, Jr. Foundation Publications, 1952, pp. 113-129.

11. Freeman, D. J.: Some Observations on Calcifying Cartilage Matrix, Thesis, University of Wisconsin Medical School, 1952.

12. Fawns, H. T., and Landells, J. W.: Application of Collagenase and Hyaluronidase to the Study of Cartilage in Histological Sections, J. Physiol. **119**:5P, 1953.

13. Dziewiatkowski, D.: Radioautographic Visualization of Sulfur ^{35} Disposition in the Articular Cartilage and Bone of Suckling Rats Following Injection of Labeled Sodium Sulfate, J. Exper. Med. **93**:451, 1951.

14. Bélanger, L. F.: Autoradiographic Visualization of the Entry and Transit of S^{35} in Cartilage, Bone, and Dentine of Young Rats and the Effect of Hyaluronidase in Vitro, Canad. J. Biochem. & Physiol. **32**:161, 1954.

15. Freudenberg, E., and György, P.: Über Kalkbindung durch tierische Gewebe III.: Biochem. Ztschr. **118**:50, 1921.

16. Roche, J., and Deltour, G.: Sur le mécanisme biochémique de l'ossification et le rôle physiologique de la phosphatase des os, Compt. rend. Acad. d. sc. **216**:748, 1943.

17. Moog, F.: Physiological Significance of the Phosphomonoesterases, Biol. Rev. **21**:41, 1946.

18. McKelvie, A. M., and Mann, F. C.: Role of Alkaline Phosphatase in Osteogenesis After Transplantation of Bone, Proc. Staff Meet. Mayo Clin. **23**:449, 1948.

19. Robinson, R. A., and Watson, M. L.: Electron Micrography of Bone, in Transactions of the Fifth Conference on Metabolic Interrelations, New York, Josiah Macy, Jr. Foundation Publications, 1953, p. 72.

20. Neuman, W. F.: Bone as a Problem in Surface Chemistry, in Transactions of the Second Conference on Metabolic Interrelations, New York, Josiah Macy, Jr. Foundation Publications, 1950, p. 32.

21. Hendricks, S. B.: Comments on the Crystal Chemistry of Bone, in Transactions of the Fourth Conference on Metabolic Interrelations, New York, Josiah Macy, Jr. Foundation Publications, 1952, p. 185.

22. Cobb, J. D.: Relation of Glycogen, Phosphorylase, and Ground Substance to Calcification of Bone, A. M. A. Arch. Path. **55**:496, 1953.

23. Greep, R. O.; Fischer, C. J., and Morse, A.: Alkaline Phosphatase in Odontogenesis and Osteogenesis and Its Histochemical Demonstration After Demineralization, J. Am. Dent. A. **36**:427, 1948.

24. Engström, A.: Microradiography, Acta radiol. **31**:503, 1949.

25. Clemmons, J. J.; Lalich, J. J., and Angevine, D. M.: Technic of Quantitative Historadiography, Lab. Invest. **3**:19, 1954.

26. Follis, R. H., Jr.: Studies on the Chemical Differentiation of Developing Cartilage and Bone: I. General Method, Alkaline Phosphatase Activity, Bull. Johns Hopkins Hosp. **85**:360, 1949.

News and Comment

ANNOUNCEMENTS

Fellowship in Dermal Pathology.—This fellowship, sponsored by the American Academy of Dermatology and Syphilology, provides opportunity for study in dermal pathology to a postgraduate student who has completed at least one year, preferably two, of training in dermatology. The stipend is $4,000 a year. A period of training will be spent at the Armed Forces Institute of Pathology, Washington, D. C., the appointment being subject to approval by the director of the institute. The American Board of Dermatology and Syphilology has approved the institute for one year of training, but the student must complete one year of graduate training either before or after completion of the Osborne fellowship, in an institution approved by the board for three years of training. Application blanks may be obtained from Dr. Hamilton Montgomery, chairman of the committee on pathology of the American Academy of Dermatology and Syphilology, 200 First St. S. W., Rochester, Minn. The next available appointment begins July 1, 1957. Early application is urged, and, as a rule, applications will not be considered after Sept 1, 1956, for the July 1, 1957, appointment.

Medical Student Research Fellowships.—Medical Student Research Fellowships, available to medical schools throughout the United States and Canada, are now being offered for the current year by Lederle Laboratories Division, American Cyanamid Company.

The Fellowships, not exceeding $600 for any one person, are intended to relieve some of the financial burden of students who desire to devote their summer vacations to basic research in the preclinical medical sciences.

Selection of students to receive the award will be made by the dean of the medical school or his selection committee. Students who apply must be of good scholastic standing and have the consent of the faculty member under whose supervision their research is to be conducted. Such research may be carried on in another medical school, if the arrangements are satisfactory to faculty authorities in both schools.

Time-Intensity Factors in Radiation Response

I. The Acute Effects of Megavolt Electrons (Cathode Rays) and High- and Low-Energy X-Rays with Special Reference to the Brain

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With the Assistance of Barbara L. Brown, B.A., and Charlotte E. DeHarport, B.S.

Advances in nuclear science in the past decade have centered new interest in what ionizing radiations may do to the brain. The effects of conventional x-rays on the brain and other tissues have seemed to be well established, but a scattering of observations has shown that changing the quality or the intensity of the radiation may alter the response considerably. When injury occurs after ordinary therapeutic radiation of the brain or cord, it is primarily the result of damage to blood vessels.[1] Changing the character of the radiation by using 32 million electron volt (mev) x-rays, Arnold, Bailey, and Laughlin [2] showed that primary destruction of the parenchyma in man and primates can occur independently of vascular damage. Haymaker [3] and others also demonstrated acute destructive lesions in the brains of monkeys following large doses of high-intensity gamma rays. Earlier Hicks and Montgomery [4] reported acute necrosis of oligodendroglia, certain neurons, and retinal cells in rats and mice following conventional x-rays, and in other studies Lyman,[5] Davidoff,[6] Nemenow,[7] and others described parenchymal damage after radiation.

In addition to specific tissue damage, interest has also turned to the immediate impairment of function, or death, that may follow heavy irradiation of the brain. Loutit * perhaps first observed convulsions and death in rabbits shortly after very large doses of ionizing radiation. In a recent preliminary report, Hicks and Wright [8] compared the acute effects of megavolt electrons (cathode rays), high voltage x-rays, and conventional x-rays on the central nervous system. They found that the speed at which radiation was given was an important factor in modifying the response to injury both in terms of tissue destruction and physiologic response. Very high intensity megavolt electrons could not only destroy gray and white matter of the brain, but they could also kill the animal instantly when the dose was high enough.

As a result of this background of observations, a joint program has been undertaken to learn how the pathologic and physiologic effects of radiation may change when certain physical factors, especially the intensity, of the radiation are varied. The possible combinations of dose, kind of radiation, and the rate at which it is given are theoretically limitless, but the ones used in these experiments were considerably determined by what was available and practicable. The striking functional and anatomic changes in the brain that result from megavolt electrons made it desirable to center attention on this form of radiation first. For compari-

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Departments of Pathology, New England Deaconess Hospital and Harvard Medical School; Department of Electrical Engineering, Massachusetts Institute of Technology, and Department of Radiology, New England Deaconess Hospital.

* Loutit, J. A.: Unpublished data, Atomic Energy Establishment, Harwell, England.

son, the acute effects of x-rays given at several voltage levels and at slow and fast rates were also studied.

This report tells of these experiments. Emphasis is on the central nervous system, but the effects on the other tissues are considered. It is expected that subsequent experiments will deal with even more diverse forms of radiation and some given at extraordinarily high rates.

MATERIALS AND METHODS

Mice and some rats were exposed to 150, 250, and 400 kv. x-rays, 2.5 mev electrons, and 3.0 mev x-rays, given in various doses and at different rates. A few experiments were done with 3 and 4 mev electrons. The acute pathological and neurological changes that resulted were studied and compared, usually after 12 to 24 hours, occasionally 2 to 3 days later. The electrons were administered in a range of doses from 100 rep (roentgens equivalent physical) to 200,000 rep in one second, and some higher doses; x-rays were investigated in doses ranging from 5000 to 30,000 r at rates of 250 to 1000 r per minute at several voltages. Three mev x-rays were given at 1000 r per minute in the dose range of 12,000 to 30,000 r, and at still higher doses at a rate of 25,000 r per minute or about 416 r per second. Except in shielding experiments, the whole body of the animal was exposed. Adult male and female mice were used of C_3H, ABC, BAF_1, Swiss albino strains, and a yellow and black and tan cross. A few pregnant animals were exposed to low doses of megavolt electrons to compare their effects with those of other radiations on the fetus. Three rats were given 50,000 or 100,000 rep of electrons in one second at 4 mev. This assured sufficient penetration of the radiation so that the results could be compared with those in the mice.

For convenience, the animal data will first be stated briefly, then the pathologic procedures and details of the physical factors will follow.

Megavolt Electron Experiments.—A total of 170 mice were used in the 2.5 mev electron experiments, distributed fairly evenly by sex and strain over the dose range. Investigated were 100, 150, 200, 300, 400, 1000, 2000, 5000, 10,000, 20,000, 30,000, 40,000, 50,000, 100,000, and 200,000 rep each given in one second. Three to six animals, occasionally more, were used in each dose level up to 20,000 rep, and pathologic studies were carried out. At 40,000 rep and above, death was usually rapid or instantaneous (to be described). At 30,000 rep some animals died soon after exposure, but 12 survived 24 hours to be killed for autopsy. A few animals were given 1,000,000 rep in 10 seconds to confirm the rapidly lethal effect of the lower doses.

Additional experiments were carried out to investigate what parts of the body, head, and brain were most vulnerable to the acute effects of the radiation. This was done by selective shielding described later.

Lower Voltage X-Ray Experiments.—Forty-five mice, mostly C_3H of both sexes, were exposed, total body, to 10,000, 20,000, or 30,000 r of x-rays. Voltages included 150, 250, and 400 kv., and rates were 250, 500, 600, or 1000 r per minute (see below). For further comparison, data were used from previously reported rats and mice exposed to doses of 5000 to 20,000 r given at 55 and 75 r per minute at 250 kv.

High Voltage (3.0 Mev) X-Ray Experiments.—For comparison with electron and lower voltage x-ray findings, the whole bodies of 24 male and female mice were exposed to 12,000, 20,000, or 30,000 r of 3.0 mev x-rays at 1000 r per minute. They were male and female BAF_1 C_3H, and Swiss albino.

Some additional experiments, without microscopic autopsy, were carried out with 10 mice exposed to these 3 mev x-rays given at a rate of 25,000 r per minute (or 416 r per second). Doses were 10,000, 20,000, 40,000, 60,000, and 100,000 r.

Other Experiments.—Two pregnant mice near term were exposed to 100 r in six seconds (1000 r per minute rate) of 250 kv. x-rays, 15 ma., no added filter, 15 cm. Five others, bellies turned upward, received 100, 200, or 300 rep of 2.5 mev electrons in one second. These were done to compare the effects on the fetus with those produced by conventional x-rays.

Pathologic Methods.—Histologic studies were carried out by fixing appropriate tissues from the gross autopsy in formalin or Bouin's fluid, embedding in paraffin, and staining with hematoxylin and eosin. Complete microscopic autopsies were performed

in three or more representative animals in all experiments. In the megavolt electron experiments some autopsies took the form of frontal sections through the whole body in order to study microscopically the gradient effect of the rays. Where complete autopsies were not carried out, the brain, eyes, a cross section of spine, small intestine, thymus, and gonads were examined histologically.

Physical Data.—The animals were exposed to the radiation either in small ventilated cardboard boxes, 2×8×8 cm. with 1 mm. thick walls, or they were enclosed in commercial household flexible plastic 16-mesh screen.

from a General Electric Maximar 250 kv. unit, while a 400 kv. unit was used for that voltage. Doses were recorded at the target with a Victoreen condensor V-meter and included back-scatter. The factors for each group of experiments were as follows: 150 kv.: 15 ma., 2 mm. Al filter added, 0.15 mm. Cu half value layer (HVL), target distance to top of mice 18 cm., 1000 r per minute, doses of 1000, 5000, and 10,000 r. 250 kv.: 15 ma., no added filter, 0.3 mm. Cu HVL, target 35 cm. for 250 r per minute, 25 cm. for 500 r per minute, and 15 cm. for 1000 r per minute, doses 10,000 and 20,000 r. 400 kv.: 5 ma., 1.25

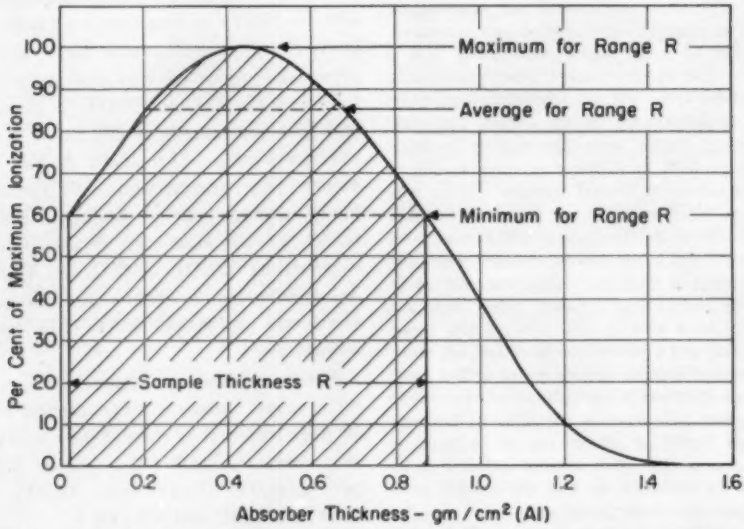

Fig. 1.—Ionization-in-depth curve for 2.5 mev electrons. It is applied to an 0.82 gm/cm.² sample of aluminum from which maximum, average, and minimum doses can be determined. The aluminum serves as a reference standard from which doses for other materials including tissues can be extrapolated. In these experiments the average dose delivered to the mouse is given. The maximum ionization occurs at a level corresponding to about one-third of the distance traveled by the electrons (see text).

The x-ray experiments in the lower voltage range were carried out primarily to learn at what level oligodendroglia, cerebral neurons, and cerebellar granule cells first showed damage, or to determine the lowest level at which neurologic symptoms and severe damage might develop. Extensive experiments at each dose and rate level were therefore not done, but in aggregate they were useful. X-rays of 150 and 250 kv. came

mm. Cu HVL, no added filter, target 12 cm., 600 r per minute, doses of 10,000, 20,000, and 30,000 r per minute. The variation was probably similar in magnitude for corresponding target distances to those given below for 3 mev x-rays.

In the experiments with high-energy monoenergetic electrons (or cathode rays) and high-energy, high-intensity x-rays, a Van de Graaff electrostatic accelerator was

employed. This equipment has been described elsewhere.[9]

In the use of high-energy electrons, a uniform transverse distribution may be obtained but a nonuniform depth distribution results for thick sections. Figure 1 is an example of the depth-dose distribution which one would obtain for 2.5 mev monoenergetic electrons. The curve shows the per cent of maximum ionization at any level in the total depth of penetration of the electrons. The aluminum block, weighing 0.82 gm. and 1 cm. square in cross section to the beam, serves as a convenient reference standard from which maximum, minimum, and average doses may be calculated for other substances including biologic materials. In these experiments the doses given represent the average, calculated for the animal from this figure. The methods for computing doses from voltage, current, time, and distribution, and for deriving the curve are outlined by Trump.†

Similar distributions, but with different ranges, would be obtained for other energies in the range from 1 mev to several mev. A uniform transverse distribution has been obtained by using a conveyor belt with the central section of the beam, in the direction of movement of the sample, partly blocked so that the time-integrated dose is uniform across the sample. In this series of experiments, the conveyor belt speed was adjusted so that the exposure time was approximately one second and the current was varied to give doses from 100 to 100,000 rep.

The same equipment was used to generate 3 mev x-rays [10] by introducing a gold target with an equivalent inherent filtration of 10 mm. of lead. However, the animals were exposed in a stationary position, which resulted in a dosage that was not uniform. The mice were considered to be 2 cm. thick, and the dose was computed to be an average one through this section. The target distance was 30 cm. for the 1000 r per minute experiments, and 7 cm. for those of 25,000 r per minute. At the 1000 r per minute rate the variation was estimated to be plus or minus 10%; at 25,000 r per minute, with the short target distance, plus or minus 25%.

Shielding Experiments. — The megavolt electrons at 20,000 to 30,000 rep per second caused severe nervous system damage in mice, and from 40,000 rep upward death ensued with increasing rapidity after exposure. At 100,000 rep death occurred very quickly, usually "instantly," that is, the animal was dead before some 10 to 20 seconds after radiation. After somewhat lower doses, such as 40,000 rep, the animal often became wabbly, then convulsive within minutes or a little longer before it died, a syndrome to be described later. Several experiments were devised to determine what parts of the nervous system, when radiated, were responsible for the rapid death and for the neurologic symptoms. Aluminum shields of suitable thickness and thin flexible lead were arranged to cover and immobilize certain divisions of the body and nervous system and expose others. Combinations included shielding the whole head and exposing the whole spine and body, shielding the midbrain-medulla-cerebellar region (brain stem) while exposing the forebrain and body, and the converses of these arrangements.

RESULTS

These findings can best be presented in general descriptive terms by dose levels and type of radiation. The effects on the nervous system, especially those of megavolt electrons, will be emphasized, but findings of interest in other organs will be noted. Illustrations of representative lesions are shown in Figures 2 through 7.

Megavolt Electron Experiments. — The first acute pathologic changes in adult mice exposed to megavolt electrons were seen at 100 to 200 rep given in one second. This was necrosis of the subependymal "embryonal" cells of the brain, which are present in young

† Reference 9. In these experiments 1 rep is taken as equal to an average energy absorption of 83 ergs per gram. For water, a dose of 10^6 rep will give a temperature rise of approximately 2 C.

TIME-INTENSITY FACTORS—RADIATION RESPONSE

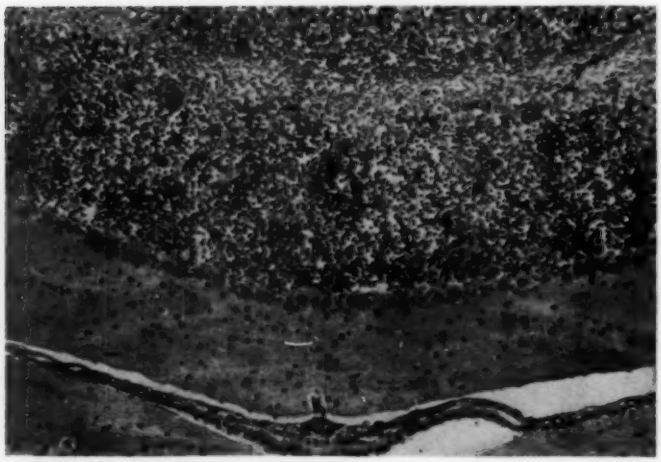

Fig. 2.—Acute necrosis of granule cells in the cerebellum caused by 100,000 rep of 2.5 mev electrons. This degree of necrosis was sometimes attained after 20,000 rep. Hematoxylin and eosin; reduced about ⅔ from mag. × 225.

adult mice and rats. This is similar to that following conventional x-rays.[1] In fetuses, the usually radiosensitive differentiating embryonal cells were extensively necrotic throughout the brain after 100 and 200 rep, indistinguishable from those following 100 to 200 r of conventional x-rays.[1] However, fetuses lying most dorsal in the mother's abdomen showed less damage. (This experiment was carried out with the pregnant animal on its back.) In the small intestines of nonpregnant animals given 150 rep there were a few scattered necrotic epithelial cells in the depths of the mucosal crypts in loops of bowel closest to the backbone. Lymphoid tissue (in thymus, spleen, intestine, lymph node) was unaffected, and bone marrow (vertebra and rib) showed no change or slight capillary engorgement. Some animals of this group were allowed to survive 4 days, and in these the radiation changes evident in 24 hours had largely disappeared and no new ones had appeared.

At 24 hours after 300 and 400 rep, the characteristic changes in the intestine and subependymal regions of the brain were slightly more evident. Ova, spermatogonia, and spermatocytes showed no measurable change. Vertebral marrow showed capillary hyperemia and rare necrotic blast cells. Few

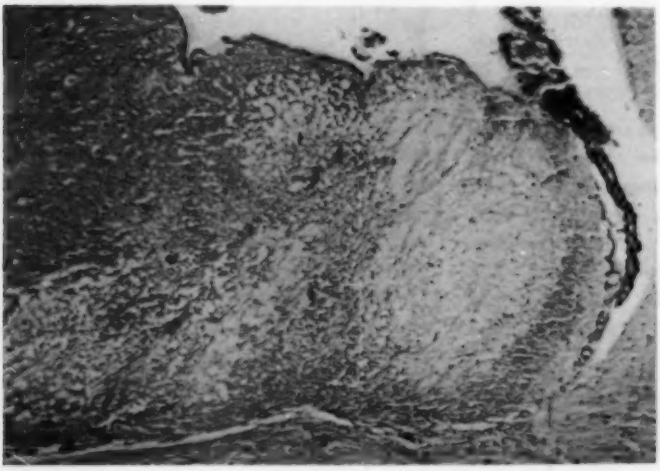

Fig. 3.—Acute necrosis of white matter and its associated oligodendroglia caused by 20,000 rep of 2.5 mev electrons. The affected glia are seen as mere black dots in the disintegrating white matter. Hematoxylin and eosin; reduced about ⅔ from mag. × 100.

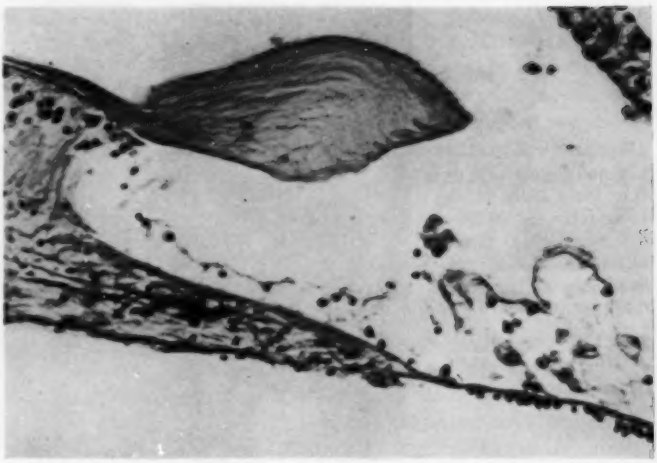

Fig. 4. — Necrosis of cells and swelling of membrane in the organ of Corti that resulted from 20,000 rep of 2.5 mev electrons. Hematoxylin and eosin; reduced about 2/5 from mag. × 575.

to moderate numbers of lymphocytes in the thymus and spleen were necrotic. The dorsal spine marrow in a pregnant animal (irradiated belly-side-up) was unaffected, but, even here, deeply situated fetuses showed slight damage. As the electron dose spectrum was ascended, the usually radiosensitive tissues (hemopoietic, lymphoid, and intestinal and subependymal brain cells) were increasingly damaged. At 24 hours after 1000 rep, where four animals were studied by whole-body frontal sections, the spinal marrow showed necrosis of most immature forms, polymorphonuclear leucocytes were frequently necrotic, and hyperemia was marked. Megakaryocytes seemed unaffected. Dorsally situated lymph nodes of the neck showed severe damage, whereas spleen, intestine, and other marrow varied a little in effects depending on the distance from the animal's back. In the head, at this dose, no certain gradient could be seen in the marrow between the vertex of the skull and the jaw. At 1000 rep, the first changes in the skin and its appendages appeared, namely, pyknosis of nuclei in the inner half of the epidermis dorsally and necrosis of rare cells just inside the basal layer of hair follicles of the chin

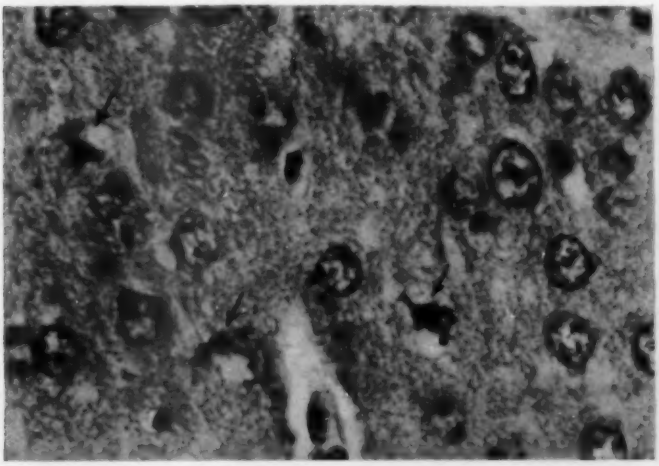

Fig. 5.—Three necrotic neurons are shown, the result of 20,000 r of 250 kv. x-rays. Hematoxylin and eosin; reduced about 2/5 from mag. × 1350.

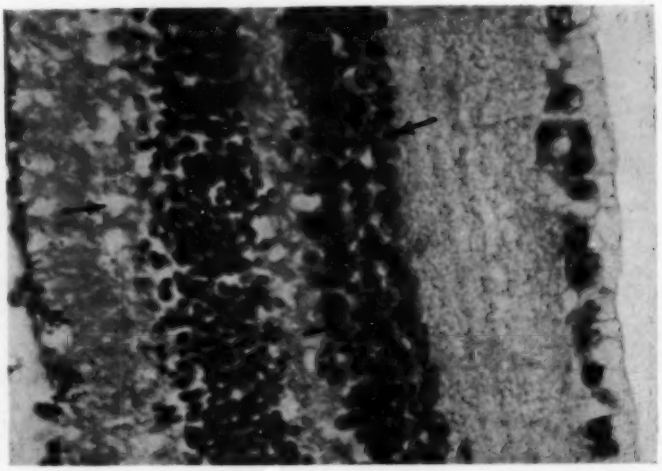

Fig. 6.—Swelling of rod cells and necrosis of nuclear layer cells in the retina following 20,000 r of 3 mev x-rays. Hematoxylin and eosin; reduced about ⅝ from mag. × 750.

whiskers. At this level oligodendroglia cells in the brain were necrotic in rare instances. In previous studies with conventional rates of 250 kv. radiation, this was first noted at the probably comparable level of 1500 r.[1] At 2000 r necrotic oligodendroglia cells were more frequent but still widely scattered. Five animals were examined by the whole-body section method. The parenchymal epithelial cells of the salivary glands showed more granularity than normal. Nuclei in the cells of the anterior pituitary, ova, and spermatic cells were pyknotic, but this was not surely greater than in some control material. Differences in effect on dorsal and ventrally situated organs and tissues were more noticeable than at 1000 r, as might be expected.

After 5000 rep, four animals were studied in whole sections. Three were autopsied at 24 hours, and one was allowed to live 4 days. The usual radiosensitive tissues showed moderate to severe damage. Respiratory epithelial cells had some clumped cilia. The animal allowed to live four days showed regeneration of intestinal crypt epithelium with characteristic cells having large nuclei and slightly basophilic cytoplasm. The spermatogonia were diminished. Scattered necrotic oligodendroglia were seen in the brains and cords of all four animals. In addition, necro-

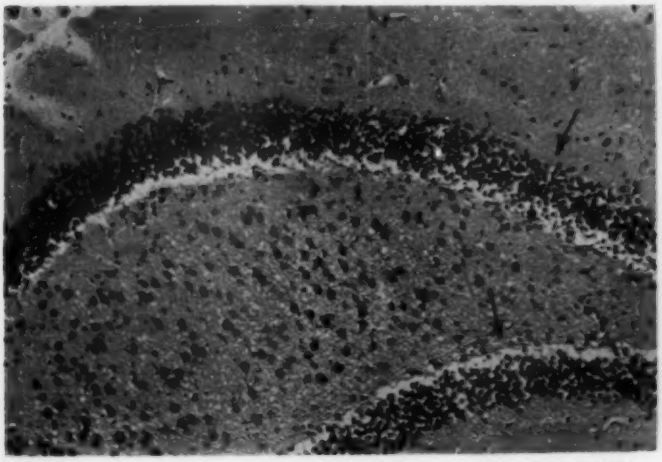

Fig. 7.—Necrosis of fascia dentata neurons in the hippocampus following 30,000 r of 3 mev x-rays. In the left half of the upper band the cells are relatively unaffected. Hematoxylin and eosin; reduced about ⅝ from mag. × 200.

sis of granule-cell neurons of the olfactory lobe was seen in one 24-hour animal, and another showed necrosis of a few cerebellar granule-cell neurons. This was the lowest level at which this cerebellar change was seen in any experiments. (This type of necrosis is illustrated in Figure 2, in which a marked degree of damage has resulted from 100,000 rep.) At 10,000 rep, although no animals showed gross neurologic symptoms, olfactory granule-cell neurons were considerably damaged and fairly numerous, but scattered oligodendroglia in the brain and cord were necrotic. Three of four animals showed a moderate degree of necrosis of granule-cell neurons of the cerebellum, particularly the central folia of the vermis. In the fourth animal pyknosis of the nuclei of these cells occurred. In one animal the rod cells of the retina, but not the nuclear layer, were necrotic. Intestinal lesions varied from moderate to no effects as the distance from the animal's back increased. Respiratory cell changes were similar to those after 5000 rep. Hemopoietic tissue within range of the radiation was moderately to severely damaged. The spermatogonia and Sertoli cells showed swollen nuclei, and a few spermatocytes were necrotic in one animal; these changes were scant in another male, but there was necrosis of some lining epithelial cells of the epididymis. Pituitary, parathyroids, thyroid, pancreas, and ovary were not remarkable except for swelling of nucleoli in ova. The innermost zone of adrenal cortical cells showed necrosis in one animal, and fresh necrosis of very rare skeletal muscle cells in the perispinal muscle groups was noted in another. Swelling of duct cell nuclei and granularity of parenchymal cell cytoplasm of salivary glands were observed. Occasionally, the stellate cells in the pulp of incisor teeth were necrotic.

At 20,000 rep, three animals were completely autopsied, and in nine the nervous system and the usually "radiosensitive" organs were studied. These animals had become severe neurologic cripples within two to three hours after radiation. Central nervous system damage, though variable in degree, was notable. In one animal, 20 hours after radiation, not only were the cerebellar granule-cell zones extensively necrotic but widespread necrosis of neurons, oligodendroglia, and white matter throughout the forebrain was present. The white matter damage was principally to the striatum and hippocampal commissures, less in the corpus callosum and other regions. Where this acute disintegration of the myelinated fibers took place, it was accompanied by necrosis of all the associated oligodendroglia cells, not just scattered ones. Figure 3 shows this damage at low power, and the necrotic oligoglia in the swollen disintegrating area appear as black dots much smaller than their normal counterparts elsewhere. This relationship was similar to that previously described for "demyelinating" lesions following poisoning with cytochrome oxidase inhibitors such as azide and cyanide.[1] The neuron necrosis was notable throughout the cortex and especially in the fascia dentata of the hippocampus, but less in the striatum and relatively inconspicuous in the brain stem. There was no vascular endothelial or thrombotic disease in association with this widespread damage. The other mice showed less forebrain damage: Neuron destruction was scattered, and oligodendroglia necrosis was fairly widespread, but not severe enough to be associated with demyelination. The cerebellar granule cells were damaged in three animals, but in one it was scant. Some epithelial cells of the organ of Corti and semicircular canals and some cells of the nuclear layer of the retina were necrotic. Cellular edema accompanied these changes (Fig. 4). Changes in other organs were similar to those seen after 10,000 rep, but severer. In the testes the spermatogonia and Sertoli cells were sometimes markedly swollen.

Thirty thousand rep was the level at which animals sometimes died very quickly after radiation. All survivors had severe neurologic symptoms ranging from complete inability to stand up to some capacity to stagger and crawl about. When stimulated, they

often thrashed about. Abbreviated protocols of 12 of these surviving animals, describing their appearance at the time of autopsy and microscopically, serve well to illustrate the changes produced by rapidly administered megavolt electrons. Severe neurologic incapacity was fully developed within two to three hours after radiation in these animals, although some sluggishness or holding the head slightly to one side was often seen immediately after radiation. They were killed 17 to 24 hours after exposure.

ANIMAL 1.—BAF_1 male, incoordinate, unable to stand or walk. Many scattered necrotic cortical neurons ‡ and necrotic oligodendroglia and patchy swelling of white matter throughout neuraxis; patchy necrosis of fascia dentata and pyramidal cells of hippocampus; necrosis of third ventricle ependymal cells; necrosis of some rod and nuclear layer cells of retina; midbrain and medulla less damaged, but granule layers of cerebellum severely damaged.

ANIMAL 2.—BAF_1 male, wobbly gait. Less severe neuropathologic picture than that in Animal 1, but as diffusely distributed. Cells of the nuclear layer of retina frequently necrotic, rod cells spared.

ANIMAL 3.—BAF_1 male, thrashing about when stimulated, totally incoordinate. Similar brain findings as those in Animal 1, but olivary nucleus and dorsal central medulla neurons frequently necrotic, rare necrotic Purkinje cells, and supraoptic nucleus neurons.

ANIMAL 4.—C_3H male, wobbly gait. Same neuropathologic pattern and severity as that of Animal 1. (Congenital absence of rod cells, common in C_3H mice.)

ANIMAL 5.—Swiss albino female, very incoordinate. Neuropathologic picture similar to that of Animal 1, but less damage in forebrain.

‡ The dead neurons here and in other descriptions appeared similar to those that result from injury by anoxia, hypoglycemia, cyanide, ischemia, etc. The cytoplasm is eosinophilic, much reduced in volume, and its margins indistinct and rarely vacuolated. The nucleus is much reduced with indistinct borders, and either homogeneously basophilic or pale and granular. The indistinct outlines of nucleus and cytoplasm help to distinguish dead cells from artifactually shrunk or reversibly injured cells. Necrotic oligodendroglia and cerebellar granular cells show similar nuclear changes, but the eosinophilic cytoplasmic change is often difficult to see because of its small volume in these cells. See photomicrographs.

ANIMAL 6.—C_3H female, similar to Animal 1 in behavior. Milder damage in cerebellum, but severe in hippocampus.

ANIMAL 7.—BAF_1 male, shaky in all movements, but mildest symptoms of any of these mice. Mild version of cerebral damage described in Animal 1. Granule cells of central cerebellar folia fairly severely damaged, but retinas unremarkable.

ANIMAL 8.—BAF_1 male, incoordinate gait, recurrent rapid thrashing and convulsive episodes when stimulated. Destructive damage in brain similar to that of Animal 1 with much oligodendroglia necrosis and edema, but neuron necrosis relatively mild; cerebellar granule cells moderately damaged.

ANIMAL 9.—BAF_1 male, very incoordinate movements, scarcely able to stand. Damage largely limited to oligodendroglia, relatively few neurons. Cerebellar vermis shows usual granule-cell necrosis.

ANIMAL 10.—ABC male, moderately incoordinate movements. Numerous scattered necrotic oligodendroglia, moderate cerebellar granule-cell and olfactory granule-cell damage, rare damage to other neurons. Rod cells undamaged, nuclear layer shows some necrosis.

ANIMAL 11.—Swiss albino male. Altogether similar to Animal 10.

ANIMAL 12.—BAF_1 female. Altogether similar to Animals 10 and 11.

Additional findings of interest in these 30,000 rep animals were severe damage to the usually radiosensitive tissues and marked cellular and intercellular testicular edema with necrosis and aggregation of degenerating spermatocytes in some. Other changes were similar to those seen after 20,000 rep. In one animal central adrenal cortical cells, and in another, rare anterior pituitary cells, apparently the eosinophiles, were necrotic. Occasionally epithelial cells of the semicircular canals and of the cochlear duct membrane were necrotic, but in some animals there was no destruction. Supporting stroma of the organ of Corti contained some necrotic cells, but pyknosis of nuclei in the adjacent ganglion neurons was not distinguishable from that sometimes seen in controls. The dorsal skin showed necrosis of cells of the outer layers of hair follicles. In the marrow of dorsal bones and in the spleen, virtually the only surviving hemopoietic cells were reticular cells and megakaryocytes. The latter showed more than usually pyknotic nuclei.

Only one animal survived 40,000 rep to be autopsied 24 hours later. The pathologic picture was essentially the same as that after 20,000 and 30,000 rep. The retina was more edematous, necrotic skeletal muscle fibers were scattered, but easy to find. The cochlear duct membrane and semicircular canal epithelia had eosinophilic lobular material on their surfaces in places. The exposed skin was a little more severely injured than at the lower doses.

When mice were exposed to 100,000 rep of electrons at 2.5 or 3.0 mev in one second, they died virtually instantly. Death had occurred by the time the exposure room could be entered after the experiment, something of the order of 10 to 20 seconds. At 50,000 rep, death was similarly rapid or delayed a minute to a few minutes. The animal suddenly burst into a running fit. Then rigid extension of limbs and body, with head flexed, followed quickly, and death was almost immediate. The fit was sometimes reminiscent of those seen in monofluoracetic acid poisoning, except that the heart usually, but not always, stopped soon after the rigor, and the rigidity of muscles was less marked. (A number of animals were autopsied promptly at this stage, as described below under "Comment.")

Forty mice were shielded in several ways, as indicated above, and exposed to 100,000 and 200,000 rep per second. If the head from the occiput forward was shielded, with the brain stem exposed, death from 100,000 rep was "instant." Shielding the whole body and head except the brain stem was similarly lethal. On the contrary, when the brain stem region alone was shielded with the anterior head, spine, and body exposed to 100,000 rep, instant death did not occur. The animal survived from 12 to 20 hours, becoming weak, wobbly, then dying. In other experiments an attempt was made to exclude the central brain stem from the field of radiation but expose the temporal bones containing the internal ears. These did not precisely succeed, but inadvertently provided very useful histologic information about what parts of the brain may be most vulnerable to radiation. Five animals were exposed to 100,000 r or 200,000 rep in one second. In two, the animals obviously wriggled well out of position and were killed instantly. Three others survived to become moribund in six hours, at which time they were killed and autopsied. The pathologic picture, quite clear-cut, revealed that these animals, too, had wriggled the anterior parts of their heads out of position but had shielded their medullas as far forward as the level of the widest part of the fourth ventricle or the upper end of the inferior olives. Anterior to this level, two animals showed intense diffuse damage to the whole forebrain and anterior brain stem. In a third animal, the shield provided protection for a part of the forebrain; otherwise the picture was the same as those of the first two. The shielded region appeared as a "shadow" of histologically normal brain in the anterior part of one cerebral hemisphere, forming a diagonal band down and across it. The damage to these forward parts was represented by widespread necrosis of neurons everywhere. Those cells that were not obviously necrotic showed swollen nuclei and cytoplasm. Often the matrix and white matter showed patchy edema and beginning disintegration. The organ of Corti and semicircular canals, anterior third to half of the cerebellum, and its most lateral folia were destructively damaged. As might be expected, these animals were unable to stand and walk a few minutes after radiation. With what was to be tantamount to destruction of the whole forebrain and midbrain, they rapidly became worse, remaining in a constant state of ineffectual wriggling and minor convulsions until killed.

The three rats exposed to 50,000 and 100,000 rep at 4 mev behaved about like 30,000 rep mice. Death was delayed to 15 to 45 minutes, and they had fits similar to those of the mice. These were done simply as check to exclude gross species differences.

Lower Voltage X-Ray Experiments.—Three mice were given 10,000 r and three were given 20,000 r at 500 r per minute of

250 kv. x-rays. Six mice received 20,000 r 250 kv. x-rays at 500 r per minute, and three more received the same dose at 250 r per minute. Eleven mice received 10,000, 20,000, or 30,000 r at 600 r per minute at 400 kv. and six received 1000, 5000, or 10,000 r at 1000 r per minute at 150 kv. All but two survived to be studied pathologically, usually 24 hours later. At 1000 to 10,000 r, the brain changes were mild and similar to those previously reported for conventional x-rays.[1] In one animal given 10,000 r at 150 kv. rare granule cells in the cerebellar vermis were necrotic, but no symptoms were observed. Retinas and internal ear structures were not damaged, but scattered oligodendroglia and some olfactory granule-cell neurons showed usual necrosis as low as 1000 r. Usually radiosensitive tissues were damaged to a degree comparable to that seen in corresponding electron experiments. At 20,000 r, damage to granule cells in the cerebellum became more conspicuous, and rare to scattered cortical and striatal neurons were necrotic (Fig. 5). In two animals, one at the 400 kv. level, the other at the 250 kv., 1000 r per minute level, a fairly severe degree of necrosis of neurons throughout the striatum was seen. The nuclear cell layer of the retina often showed scattered necrotic cells. The 400 kv. animals showed damage to other tissues comparable to those caused by corresponding doses of megavolt electrons. Except for the striatal damage noted, the degree of brain damage was generally less than that after comparable megavolt electrons. After 30,000 r of 400 kv. x-rays the brain damage was similar to that seen after 20,000 r, one animal showing marked damage to neurons in the striatum, the others a good deal less. Injury to other tissues, including the deep crypt cells of the small intestine, was as severe as after 20,000 r or rep levels. Cells of the islets of Langerhans were occasionally to completely necrotic in some animals.

Mice receiving 20,000 r or 30,000 r became incoordinate, unable to walk in about three hours, and often thrashed about if disturbed. Two mice in each group of the 250 kv., 20,000 r experiments died in less than 24 hours, but convulsions and fits were not observed. These dead animals were in addition to those studied pathologically.

The effects of 250 kv. x-radiation on the fetuses that received 100 r in six seconds (1000 r per minute rate) were indistinguishable from those following comparable doses of megavolt electrons or x-rays at conventional rates.

High Voltage (3.0 Mev) X-Rays.—Twenty-four mice were exposed in three groups of eight to 12,000, 20,000 and 30,000 r, all at 1000 r per minute. Brain damage after 12,000 r was generally similar to that after comparable doses of the other x-rays, and in two animals fairly substantial necrosis of cerebellar granule cells occurred. At 20,000 r, the effects were similar to those after other x-rays, usually scattered dead oligodrendroglia and occasional neurons, some destruction of cerebellar and olfactory granule cells and retinal nuclear layer cells. Though not destroyed, the rod-cell layers were swollen (Fig. 6). In one animal the number of necrotic cerebral cortical neurons was considerable, and in another, striatal neurons were frequently damaged. The appearance of neurons throughout the cortex and striatum was notable, in that cytoplasm and nuclei did not stain sharply. Though frank destruction was not widespread, this difference in sharp staining reaction from normals and from animals given 20,000 r of other x-rays was consistent. After 30,000 r four mice died within a few hours, and four survived to be autopsied the next day. Injury was severe and destruction of cortical and striatal neurons was often considerable. In one animal large segments of the pyramidal neurons of the hippocampus were destroyed, as were cortical neurons, especially the outer layers (Fig. 7). In the four animals the cerebellar granule-cell damage ranged from mild to severe.

Damage in other organs was similar to that following comparable doses of the other radiations. In all of the animals exposed to the 3 mev x-rays there was considerably less

damage to the intestines than was expected. The intestinal lymphoid tissue, however, was completely necrotic. Further studies on this apparent discrepancy are in progress.

In behavior the 12,000 r animals showed no distinctive neurologic symptoms, but the 20,000 and 30,000 r animals became wobbly, incoordinate, and able to stand with difficulty, or not at all. Although the 30,000 r and the most severely crippled 20,000 r animals thrashed and rolled about sometimes when stimulated, convulsions and fits did not occur. This aspect was further screened by exposing 12 mice, in pairs, to 10,000, 20,000, 40,000, 60,000, and 100,000 r at rates of 25,000 r per minute (416 r per second). Although one of each pair of mice was covered with ¼-inch thick masonite fiber to bring the maximum dose of the radiation up to the level of the skin, it did not visibly affect the results of the experiments. The 60,000 and 100,000 r animals lived for one to two hours, one dying in convulsions. All four became listless, weak, and then somewhat incoordinate within a few minutes. The 10,000 r animals lived two to three days without neurologic symptoms, and the 20,000 and 40,000 r animals became incoordinate in about one to two hours. No microscopic autopsies were done in this special group.

SUMMARY AND COMMENT

Mice were exposed to a wide range of doses of high-intensity megavolt electrons (cathode rays) and to x-rays given at several voltages, rates, and doses.

High-intensity megavolt electrons could cause severe brain damage or, by affecting the lower medulla, cause instant death at very high doses. X-rays of high or low voltage were less effective in these respects.

Several points of interest may be emphasized. First, megavolt electrons, which were the most studied, began to damage the cerebellar granule neurons at 5000 rep, and this became a little more evident at 10,000 rep. The degree of damage attained a maximum—necrosis of virtually all the granule cells in an affected region—in some animals that received 20,000 rep. X-rays given at the rapid rates first produced this change very slightly at 10,000 r, and it became more prominent at 20,000 r. Electrons at 2.5 mev and 20,000 or 30,000 rep not only caused considerable widespread forebrain damage but occasionally made patchy regions of necrosis in the white matter, with accompanying destruction of oligodendroglia in the affected zones. X-rays did not cause this "demyelinating" effect, although they sometimes caused substantial necrosis of forebrain neurons.

A second point of interest was the instant or relatively rapid death that followed high doses of cathode rays (40,000 rep or more). Selective shielding showed that the zone of the head and upper neck embracing the caudal half of the medulla was the critical zone. This was not only demonstrated by excluding different parts of the head from the rays by shielding and noting the physiologic effects, but by the histologic picture as well. Animals with shielded lower medullas that were given 100,000 or 200,000 rep lived long enough to be killed for study six hours later. Ordinarily these doses are instantly lethal. From the anterior medulla forward in these shielded animals, necrosis of cells and matrix of the brain was extraordinarily widespread, whereas the shielded lower medulla was unaffected. Since in these experiments the organ of Corti was severely damaged, it may be concluded that this organ is not primarily concerned with instant death. This possibility was explored because the hearing organ can be considerably damaged by radiation,[10] and because lethal convulsions can be induced in certain strains of animals by stimulating the auditory mechanism.

The precise mechanism of death needs still to be worked out further, but injury to cardiorespiratory regulating mechanisms based in the reticular substance and lower cranial nerve nuclei of the lower medulla offers a tentative explanation. A number of animals, whose lethal fit after 50,000 rep was extended over a minute to several min-

utes, were examined just after or toward the close of the episode. As described before, this episode began with a burst of running movements and was then followed by rigid extension of the body with the neck flexed. Opening the thorax and respiratory passages revealed little or no pulmonary edema, unless the process was delayed and spread over some minutes. In these delayed deaths, a little pulmonary edema developed, and the heart beat irregularly and then stopped. A few inspiratory movements sometimes occurred. At higher doses, 100,000 to 200,000 rep, cardiac and respiratory arrest occurred extremely rapidly; the animals could be recovered from the radiation room in 10 to 20 seconds after exposure. Such animals showed no pulmonary edema, and usually had never had time to develop a fit or rigidity.

Another matter of interest was the considerable damage that 3 mev x-rays could induce in the brain. The high voltage did not act as a mitigating factor in the brain. To the contrary, in the small intestine less damage was seen than expected.

In summary, the damage done by 3 mev x-rays was comparable to that following the lower voltage x-rays, but in one instance was severer. The effects of megavolt electrons at 20,000 and 30,000 rep was often considerably severer than that following x-rays. Very high doses of low voltage x-rays were not tried, but the comparison of 40,000 to 100,000 rep electrons with 40,000 to 100,000 r of 3 mev x-rays, rapidly given, showed that the former were more quickly lethal. Altogether, the intensity of the radiation, i. e., the time in which the dose was given, emerged as an important factor in determining severity of brain damage.

REFERENCES

1. Hicks, S. P.: Effects of Ionizing Radiation on the Adult and Embryonic Nervous System, A. Res. Nerv. & Ment. Dis., Proc. (1952) **32**:439, 1953.

2. Arnold, A.; Bailey, P., and Laughlin, J. S.: Effects of Betatron Radiations on the Brain of Primates, Neurology **4**:165, 1954.

3. Haymaker, W., and others: Effects of High Energy Total Body Gamma Irradiation on the Brain and Pituitary Gland of Monkeys, Official Program of the International Congress of Clinical Pathology, p. 70, 1954; in Am. J. Clin. Path. **24**: Aug., 1954.

4. Hicks, S. P., and Montgomery, P. O.: Effects of Acute Radiation on the Adult Mammalian Central Nervous System, Proc. Soc. Exper. Biol. & Med. **80**:15, 1952.

5. Lyman, R. S.; Kupalov, P. S., and Scholz, W.: Effect of Roentgen Rays on the Central Nervous System, Arch. Neurol. & Psychiat. **29**:56, 1933.

6. Davidoff, L. M., and others: Effect of Radiation Applied Directly to the Brain and Spinal Cord, Radiology **31**:451, 1938.

7. Nemenow, M. I.: Effect of Roentgen Rays on Brain, Radiology **23**:94, 1934.

8. Hicks, S. P., and Wright, K. A.: Variation of Pathologic Response with Time-Intensity Factors, Official Program of the International Congress of Clinical Pathology, p. 70, 1954; in Am. J. Clin. Path. **24**: Aug., 1954.

9. Trump, J. G.; Wright, K. A., and Clarke, A. M.: Distribution of Ionization in Materials Irradiated by Two and Three Million-Volt Cathode Rays, J. Appl. Physics **21**:345, 1950.

10. Trump, J. G., and Cloud, R. W.: Production and Characteristics of 3,000 Kilovolt Roentgen Rays, Am. J. Roentgenol. **49**:531, 1943.

11. Gerstner, H. B.; Konecci, E. B., and Taylor, W. F.: Effect of Local Brain X-Radiation on Pinna Reflex of Guinea Pigs, Radiation Res. **1**:262, 1954.

Experimental Medial Hypertrophy and Hyperplasia of Cats' Pulmonary Arteries

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In 1951, Hoff, Kell, Hastings, Sholes, and Gray [1] reported that electrical stimulation of loci on the anterior sigmoid gyrus of the cerebral cortex in acute experiments on cats evoked transient elevation of arterial blood pressure together with renal cortical ischemia. These pressor and renal vasomotor effects were greatly exaggerated when the brain was stimulated through the intact cranium with the Brief Stimulus Technique Apparatus * (B. S. T.). Following multiple stimulations of the brain with the B. S. T. apparatus in chronic experiments lasting one to six weeks, there were pathological changes in the glomeruli and tubules of the kidneys. It was observed also that in acute experiments at the height of the pressor response the lungs were engorged with blood. This was seen directly under white light and under ultraviolet light after intravenous injection of fluorescein and was further demonstrated by intracarotid injection of methylene blue or India ink at the peak of the pressor effect. In some of the chronic B. S. T. stimulation experiments, besides the renal changes, a lesion of the pulmonary vessels was also encountered. This lesion was fundamentally a medial hypertrophy and hyperplasia of the pulmonary arteries and arterioles.

There exist very few reports describing effects of chronic cerebral stimulation upon the pulmonary vascular system. In 1942, Neumann, Cohn, and Katzenelbogen [2] in studies of experimental "shock therapies" in cats with insulin and pentylenetetrazol (Metrazol) found that 5 out of 11 cats given pentylenetetrazol showed a peculiar condition of the pulmonary vessels, which they termed endarteritis. They were at a loss to explain these changes and found no reference to the phenomenon in the literature. In contrast to these experimentally produced pulmonary vessel changes in cats, the spontaneous appearance of a similar lesion has been reported by Olcott, Saxton, and Modell.[3] In the course of pharmacologic investigations, one of them (W. M.) observed on microscopic examination of sections of the lungs of more than 150 apparently normal cats that in 2 of these there was advanced hypertrophy and hyperplasia of the smooth muscle of the pulmonary arteries.

It would appear, however, that the spontaneous occurrence of this hypertrophy and hyperplasia in the feline pulmonary arteries is rare. In some species of laboratory animals the structure of the pulmonary arteries and arterioles is such as to suggest hypertrophy. In 1931, Ettinger † reviewed the literature on the anatomy of the pulmonary arteries of

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* Offner Brief Stimulus Technique Apparatus, Offner Electronics, Inc., 5320 N. Kedzie Ave., Chicago 25.

† References 4 and 5.

animals and found that the muscular coat of the pulmonary arteries of the guinea pig is not a continuous layer of plain muscle, but is interrupted at more or less regular intervals, giving it a beaded appearance resembling hypertrophy. The opossum is the only other animal in which this particular structure of the pulmonary artery has been demonstrated. Vascular disease is found in cats, and recently Lindsay and Chaikoff [6] concluded that naturally occurring systemic arteriosclerosis is common in these animals, particularly in old age, but no mention was made of pulmonary vascular changes.

The present study was undertaken to explore in further detail the medial hypertrophy and hyperplasia of the pulmonary arteries following excitation of the cerebral pressor mechanism by various stimuli. In view of the observations of spontaneous pulmonary lesions by Olcott, Saxton, and Modell, it was considered important to establish whether the changes in the pulmonary arteries of stimulated animals were of spontaneous origin or were actually produced by excitation of the brain.

EXPERIMENTAL METHODS

Animals and Programs of Study.—The experiments reported here were conducted on 57 male and female cats.

Control Series: To evaluate the frequency of spontaneous pulmonary arterial hypertrophy and hyperplasia in laboratory cats, 28 control cats were killed and multiple sections of the lungs examined for pulmonary arterial lesions. Seventeen of these animals were adults of unknown age and were killed by intramuscular injection of a lethal amount of *d*-tubocurarine. Eleven were of known age,‡ from 3 months to 4 years old, and were killed by inhalation of ether.

Pentylenetetrazol Series: In an experimental group of 19 cats, pentylenetetrazol § was administered intravenously or intramuscularly as a means of stimulating the vasomotor centers of the brain. In two of these animals, convulsive intravenous doses of pentylenetetrazol were given twice daily, and in three, a convulsive dose was administered by vein once a day. The intravenous doses ranged from 40 to 150 mg., the average being 90 mg. These five animals survived for a period ranging from 10 days in the shortest experiments to 62 days in the longest experiment. The remaining 14 experimental animals received subconvulsive intramuscular injections of pentylenetetrazol (15 to 30 mg. in each dose). Of these 14 cats, 2 received the injections twice daily, and the remainder received them four times daily. Of the 14 animals given the subconvulsive injection four times daily, 3 were kittens (littermates) 4 months old, and all 3 of these developed convulsions terminally.

Brief Stimulus Technique Apparatus Series: In another series of 10 cats, an Offner Brief Stimulus Technique Apparatus was used as the source of the stimulus, which was delivered through two 1 cm. sq. cold rolled steel electrodes. These were applied bilaterally to the freshly shaven, uncut skin of the head so as to span the pressor region of the frontal lobes. Optimum pulse duration was 0.5 msec., and the stimulus duration was 2 seconds. The peak current varied between 150 and 300 ma., and the average current, between 10 and 18 ma. for a good pressor response. The resistance between the electrodes ranged from 500 to 1000 ohms. Three animals received only this type of stimulation. Of these, one cat received only 10 stimulations at one session. The other two were subjected to a total of 50 and 55 stimulations over a period of 29 and 39 days, respectively. Each of these was given 10 to 15 stimulations at one session. A fourth cat received a total of 87 stimulations in a pattern of 10 to 15 stimulations on seven different occasions over a period of 27 days, and at the end of each session of stimulation 0.5 cc. of 1:1000 solution of epinephrine was given intramuscularly. The remaining six animals of this series received B. S. T. stimulations followed by hemorrhage of a given percentage of total blood volume, calculated on the basis of 50 cc. of blood per kilogram of body weight. In this group of six, the first animal was given 15 B. S. T. stimulations on four different occasions, and after three of these, lost 5% to 15% of the total blood volume. This animal was killed on the 33d day of the experiment. The next animal received 15 B. S. T. stimulations followed by hemorrhage of 15% of the total blood volume on one occasion only. It then developed spontaneous ataxia and convulsions and was killed on the 19th day of the experiment. The remaining four cats received on one occasion only 1, 5, 8, and 20 B. S. T. stimulations, respectively, and each was then deprived of 20% of the estimated blood volume. These animals were killed on the 59th, 119th, 32d, and 107th days of the experiments, respectively.

Postmortem and Histological Studies.—At the termination of each experiment a complete postmortem examination was performed. All organs

‡ Obtained from The Lemberger Company, supplies from Natural Store, 1436 South Park Ave., P. O. Box 482, Oshkosh, Wis.

§ Metrazol, from Burroughs Wellcome & Company, Inc.

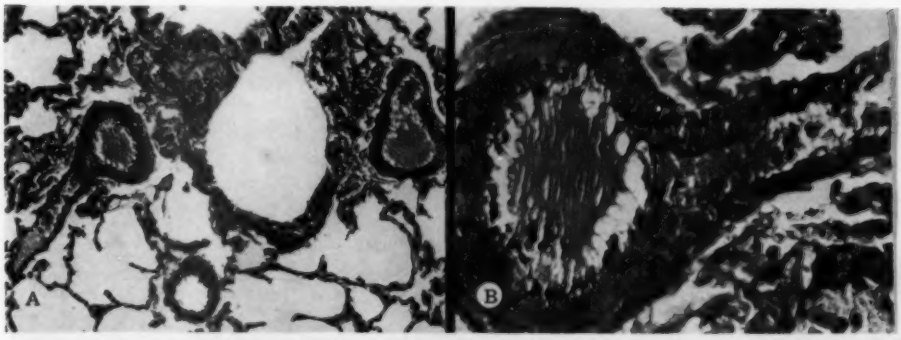


Fig. 1 (Experiment C. A. C. No. 481).—*A*, normal structure of pulmonary arteries in the lung of a 3-year-old control cat. Medium-sized pulmonary arterial walls reveal normal thickness in both transverse and longitudinal sections. Hematoxylin and eosin stain; reduced from mag. × 100. *B*, enlargement of portion of a pulmonary artery shown in *A*, illustrating normal thickness of the wall at a point of division. Hematoxylin and eosin stain; reduced from mag. × 500.

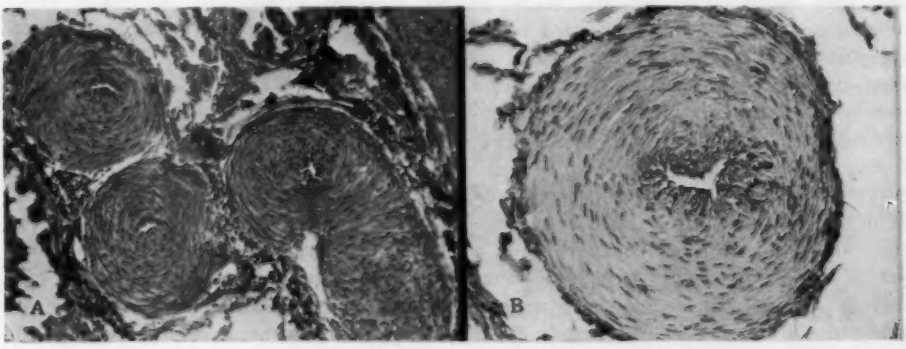
were weighed and preserved in 10% buffered formalin. Sections of the lungs and all other viscera were stained with hematoxylin and eosin, and in selected cases Masson trichrome and Verhoeff-Van Gieson stains were made of the lungs.

RESULTS

Control Series.—In the 17 control cats of unknown age, no abnormalities of the pulmonary arteries were found except in 4 animals; in the latter a slight prominence of the media was found which could be classified as minimal and was unaccompanied by intimal change. In the 11 control animals of known age (3 months to 4 years old), there was no evidence whatever of medial hypertrophy or pulmonary arteriosclerosis (Fig. 1*A* and *B*).

Pentylenetetrazol Series.—Of the 19 cats treated with pentylenetetrazol, only 4 failed to exhibit changes of the pulmonary arteries. A total of 15 revealed varying degrees of medial hypertrophy and hyperplasia (extreme in 3, pronounced in 4, moderate in 3, and slight in 5) (Fig. 2*A* and *B*). Of the three kittens that received intramuscular injections, only one showed pulmonary arterial

Fig. 2 (Experiment C. A. C. No. 282).—*A*, striking medial hypertrophy and hyperplasia of pulmonary arteries in the lung of cat subjected to intravenous injections of convulsive doses of pentylenetetrazol once a day for 49 days. Hematoxylin and eosin stain; reduced from mag. × 100. *B*, enlargement of cross section of single pulmonary artery in the lung of cat described in *A*, showing medial hypertrophy and hypertrophy and hyperplasia. Hematoxylin and eosin stain; reduced from mag. × 200.



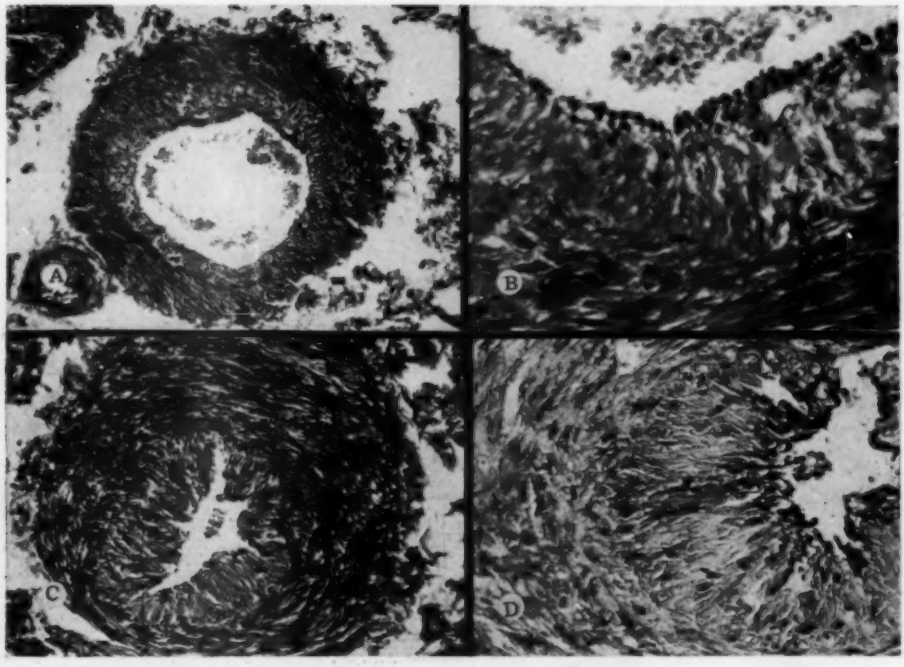


Fig. 3 (Experiment C. A. C. No. 329).—*A*, intimal proliferation in large pulmonary artery and medial hypertrophy and hyperplasia of arteriole in the lung of cat subjected to one Brief Stimulus Technique stimulation followed by hemorrhage of 20% of estimated total blood volume. Killed 59 days later. Hematoxylin and eosin stain; reduced from mag. × 100. *B*, enlargement of portion of pulmonary artery shown in *A*, illustrating intimal proliferation. Hematoxylin and eosin stain; reduced from mag. × 500. *C*, medium-sized pulmonary artery in lung of cat described in *A*, showing medial hypertrophy and intimal proliferation. Hematoxylin and eosin stain; reduced from mag. × 200. D, detail of intima of medium-sized pulmonary artery of lung of cat described in *A*, showing intimal proliferation. Verhoeff-Van Gieson stain; reduced from mag. × 500.

changes and these were of moderate degree. In only one of the animals given pentylenetetrazol was intimal proliferation found.

Brief Stimulation Technique Series.—Of the three animals that received B. S. T. stimulation alone, the cat subjected to 10 stimulations at a single session revealed no pulmonary vascular changes. The two cats given a total of 50 and 55 B. S. T. stimulations over a period of 29 and 39 days, respectively, showed slight changes in the pulmonary arteries. The animal receiving 87 stimulations in a pattern of 10 to 15 stimulations on seven different occasions over a period of 27 days plus epinephrine after each session showed no pulmonary arterial changes. Of the six cats receiving B. S. T. stimulation followed by hemorrhage, all developed moderate to extreme medial hypertrophy and hyperplasia. In addition, two of these animals showed intimal proliferation or so-called endarteritis obliterans (Fig. 3*A* through *D*). Pulmonary edema occurred in only 1 of the 29 animals of the pentylenetetrazol and B. S. T. series, this finding suggesting that the pulmonary arterial changes were not secondary to cardiac failure.

COMMENT

The pulmonary arterial medial hypertrophy and hyperplasia and the intimal proliferation seen in our experiments cannot be considered incidental findings, since they are not present in the control animals. We

believe that these pulmonary arterial changes are a chronic response of the vessels to repeated, widespread, and massive vasomotor stimulation resulting from firing of central sympathetic centers by pentylenetetrazol or B. S. T. The site of action of pentylenetetrazol upon the central nervous system lies above the level of the spinal cord, since the pressor effects of pentylenetetrazol are abolished, in our studies and those of Haury and Gruber [7] in cats, by high spinal cord transections. In dogs, Woodbury, Hamilton, Cleckley, and Volpitto [8] abolished the blood pressure rise with high spinal anesthesia.

The pulmonary arterial medial hypertrophy and hyperplasia and, secondarily, the intimal changes may be the result of one of two factors, or both. They may be caused by repeated pulmonary arterial vasoconstriction of neurogenic origin. Vasoconstriction of the pulmonary arteries has been reported by Kure, Ikeda, and Sakurasawa [9] following stimulation of the dorsal roots in dogs. However, Langford, Patterson, Porter, Bernhaut, and Hoff [10] have recently found little or no change in the pulmonary capillary, the pulmonary artery, and the right atrial pressures following stimulation of cerebral cortical pressor areas. It should be noted that the latter workers were using threshold electrical stimuli applied locally to the exposed cortex, whereas in the present studies we employed widespread convulsive and subconvulsive pharmacologic and electrical stimuli.

The other factor is the tendency for blood to be pooled in the lesser circulation during the generalized peripheral vasoconstriction associated with pressor responses to cerebral stimulation. As stated previously, this pooling has been demonstrated by Hoff, Kell, Hastings, Sholes, and Gray [1] by accumulation in the lungs of methylene blue or India ink solution injected into the common carotid artery at the height of the blood pressure rise. Possibly, these two mechanisms working together are responsible for the medial hypertrophy and hyperplasia.

Epinephrine and hemorrhage were introduced as complications of B. S. T. stimulation to enhance the systemic vasoconstriction. It would appear that hemorrhage does potentiate the pulmonary lesion. In the single epinephrine experiment pulmonary changes were not found. No explanation for this is forthcoming at present.

SUMMARY

Experiments are reported in which medial hypertrophy and hyperplasia in the pulmonary arteries and arterioles of cats were produced by chronic stimulation of suprasegmental centers of autonomic control.

This was accomplished by stimulation of the brain through the intact cranium with the Offner Brief Stimulation Technique Apparatus and by repeated chronic parenteral administration of pentylenetetrazol.

In addition, six cats received B. S. T. stimulations followed by hemorrhage. Two of these animals, one receiving one B. S. T. and the other five B. S. T. stimulations, in each case with hemorrhage of 20% of their calculated blood volume following the stimulation, revealed intimal proliferation as well as media hypertrophy and hyperplasia of the pulmonary arteries.

It is suggested that the experimentally produced pulmonary arterial medial hypertrophy and endarteritis obliterans are probably responses to repeated pulmonary arterial and arteriolar vasoconstriction or engorgement of the lesser circulation, or both.

Prof. Frank L. Apperly and Prof. Arnold R. Rich gave helpful suggestions. Mr. Melvin C. Shaffer prepared the microphotographs.

REFERENCES

1. Hoff, E. C.; Kell, J. F., Jr.; Hastings, N.; Sholes, D. M., and Gray, E. H.: Vasomotor, Cellular and Functional Changes Produced in Kidney by Brain Stimulation, J. Neurophysiol. **14**:317, 1951.

2. Neumann, M. A.; Cohn, R., and Katzenelbogen, S.: Experimental "Shock Therapies" in Cats with Insulin and Metrazol, Am. J. Psychiat. **98**:668, 1942.

3. Olcott, C. T.; Saxton, J. A., and Modell, W.: Medial Hyperplasia in Pulmonary Arteries of Cats, Am. J. Path. **22**:847, 1946.

4. Ettinger, H. G.: Investigation of the Conditions of the Pulmonary Circulation in the Guinea-Pig, Quart. J. Exper. Physiol. **21**:55, 1931.

5. Ettinger, H. G.: Action of Janus Green upon Blood-Vessels, Quart. J. Exper. Physiol. **22**:167, 1932.

6. Lindsay, S., and Chaikoff, I. L.: Arteriosclerosis in the Cat, A. M. A. Arch. Path. **60**:29, 1955.

7. Haury, V. G., and Gruber, C. M.: Action of Pentamethylenetetrazol (Metrazol) on the Circulatory System, J. Pharmacol. & Exper. Therap. **65**:227, 1939.

8. Woodbury, R. A.; Hamilton, W. F.; Cleckley, H. M., and Volpitto, P. P.: Effect of Metrazol upon the Blood Pressure of Man and Dog, J. Pharmacol. & Exper. Therap. **73**:431, 1941.

9. Kure, K.; Ikeda, R., and Sakurasawa, F.: Spinal Parasympathetics (Vasoconstrictor Effect of the Spinal Parasympathetic on Pulmonary Vessels), Quart. J. Exper. Physiol. **22**:339, 1932.

10. Langford, H. G.; Patterson, J. L., Jr.; Porter, R. R.; Bernhaut, M., and Hoff, E. C.: Hemodynamics and Acute Hypertension Produced by Stimulation of the Cerebral Cortex, Fed. Proc. **13**: 84, 1954.

News and Comment

PERSONAL

Appointment of Dr. David G. Freiman.—Dr. David G. Freiman has become pathologist-in-chief and director of laboratories at Beth Israel Hospital, Boston, and clinical professor of pathology at the Harvard University Medical School. Dr. Freiman was formerly associate professor of pathology at the University of Cincinnati.

Appointments.—Dr. Paul B. Szanto has been made Associate Professor of Pathology in the Chicago Medical School.

Elbert DeCoursey, Commandant of the Army Medical Service School at Brooke Army Medical Center, has been made an honorary member of the National Society of Anatomical Pathology of Venezuela.

Dr. Charles Phillips, formerly head of the Department of Surgical Pathology and Pathologic Anatomy at the Scott and White Clinic, Temple, Texas, has been made Associate Pathologist at the M. D. Anderson Hospital and Tumor Institute, the University of Texas at Houston.

GENERAL NEWS

"Blood Vessel Banks" and Mortuary Science.—Dr. I. M. Feinberg, of the Worsham College of Mortuary Science, Inc., has written to us pointing out the problem presented to the undertakers and embalmers from the newer emphasis upon preservation of material for "blood vessel banks." He has pointed out that many of the objections to necropsy on the part of undertakers have disappeared because of the cooperation by pathologists. If more blood vessels are removed for surgical use, this will reopen the problem from the standpoint of the undertakers. His purpose in writing was to call attention to the fact that this problem is one which will require thought and action by pathologists.

Changes in Serum Lipids and Coronary Arteries of the Rat in Response to Estrogens

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Coronary atherosclerosis is a common cause of death among young and middle-aged men, whereas women of the same age group are remarkably spared by this disease.* After the menopause, this distinct advantage of women is lost, and their morbidity rate gradually approaches that of men. These well-documented facts have led to further investigative effort, and data have accumulated supporting the importance of gonadal endocrine factors in the pathogenesis of human coronary atherosclerosis.† The prevalence of this disease among male human beings and its relative absence among female human beings during their reproductive years have led to the hypothesis that there is a deleterious factor associated with androgens, or, on the contrary, that there is a protective factor associated with estrogens. Although the bulk of the investigative work has been directed toward establishing the latter, there have been a number of apparently dissenting reports.

In 1946, Lindsay and co-workers [8] reported that they had been able to produce hyperlipemia and aortic atheromatosis in cockerels by implanting pellets of diethylstilbestrol. These workers used young adult birds, maintained them on a stock diet, and implanted 100 mg. of diethylstilbestrol over a period of 65 days. The animals were killed several months later, and they were found to have a severer aortic atheromatosis than the untreated controls. In 1948, this same group of investigators amplified their results in a more extensive study.[9] That same year, Horlick and Katz [10] showed that similar results could be obtained with use of immature cockerels. In 1950, Stamler and Katz [11] reported that the aortic lesions produced by cholesterol feeding in immature cockerels were severer in those birds receiving 1 mg. of estradiol daily than in untreated controls.

In all of these earlier studies pathologic examination apparently was confined to the aorta. Lindsay and Chaikoff [12] were the first to study the effects of estrogens on coronary atherogenesis. They injected old cockerels (33 months of age) with diethylstilbestrol and observed medial lipid deposition in the coronary arteries. The details of dosage and duration of treatment were not given. No other recorded accounts of estrogen-induced coronary atheromata have been found.

In 1951, Pick and co-workers [13] reported that estradiol was effective in prophylactically inhibiting the development of coronary atherosclerosis in immature cockerels when 1 mg. of the hormone was administered daily

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Submitted in partial fulfillment of the requirements for the degree of Master of Science in Pathology, June, 1955 (Dr. Merle S. Moskowitz).

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* References 1-5.

† References 6 and 7.

in conjunction with a cholesterol-containing diet. In a later series of papers ‡ they reported that similar doses of estradiol induced regression of cholesterol-induced coronary lesions. In all of these later studies it was concluded that there was no effect of estradiol upon aortic atherosclerosis.

There are as yet no reports of estrogen-induced atheromatosis in the mammalian species, and there has been little investigation of experimental estrogen protection in mammals.

The effect of exogenous estrogens on the blood lipid pattern, however, has been studied in the rat [17] and in man § as well as in the chicken.‖ In each instance it was similarly demonstrated that the serum lipid levels rose in response to the sex hormone, and where measured, it was shown that the phospholipid increase was proportionally greater than the cholesterol increase. Thus the net result was depression of the serum cholesterol-lipid phosphorus ratio. Lindsay and co-workers [22] have shown this effect to be reversible by simple withdrawal of the estrogens.

In one of the studies reported by Pick, Stamler, Rodbard, and Katz,[13] comparison of plasma lipid concentrations and coronary lesions revealed a close association between depression of the cholesterol-lipid phosphorus ratio and inhibition of coronary atherosclerosis. Moreover, elevation of the cholesterol-lipid phosphorus ratio has been associated with an increased incidence of coronary atherosclerosis in man,¶ the rat,# the dog,[28] and the rabbit.[29]

Neither castration [29] nor exogenous androgens * have been found to influence blood lipid concentrations in any of the experimental animals studied. However, Barr [30] has recently suggested that testosterone may be capable of producing abnormal deviations in the human plasma lipoprotein pattern, whereas in a recent report by Rossi and Giaquinto [36] testosterone has been said to cause regression of aortic atherosclerosis in the rabbit.

Preliminary studies in this laboratory † showed that estradiol enhanced the early appearance of coronary atheromata in castrated male rats maintained on an "atherogenic" diet of the type used by Wissler and co-workers.[27] The present study was undertaken to investigate further the action of estrogens and androgens on coronary atherogenesis in the rat, an omnivorous mammal, and concurrently to study associated changes in the blood lipid pattern.

MATERIALS AND METHODS

Experimental Plan

Sixty female and seventy male well-nourished, young adult rats were used in this experiment. They were individually marked for identification, weighed, and bled for serum lipid determinations. The rats were divided into nine monosexual groups (Table 1), which were comparable with respect to age, distribution of initial weights, and serum lipid concentrations. Animals in four of the groups (Table 1) were then castrated and allowed to convalesce for one week before the experiment began. At this time all groups were placed on a daily regimen of feeding via stomach tube. At the same time daily subcutaneous injections of estradiol benzoate, testosterone propionate, or sesame oil were initiated. Groups I and II were subjected only to dietary treatment and control injections of sesame oil. All other groups were treated as indicated in Table 1. Constant amounts of the same high-fat, high-choline, synthetic liquid diet, con-

Table 1.—*Animal Groupings*

Group	No. of Rats	Sex	Age, in Mo.	Wt., in Gm.*	Castration	Injections†
I	15	F	4	243	0	S. O.
II	15	M	4	378	0	S. O.
III	15	F	6	240	+	S. O.
IV	15	M	6	376	+	S. O.
V	15	F	6	240	+	T. P.
VI	15	M	6	380	0	T. P.
VII	15	F	6	238	0	E. B.
VIII	15	M	6	408	+	E. B.
IX	10	M	4	392	0	E. B.

* Mean values.
† Injections: T. P. indicates testosterone propionate in sesame oil; E. B., estradiol benzoate in sesame oil; S. O., plain sesame oil.

‡ References 14-16.
§ References 18-20.
‖ References 10, 21, and 22.
¶ References 23-25.
# References 26 and 27.
* References 30-35.

† Moskowitz, M. S., and Wissler, R. W.: Unpublished data, 1952-1954.

taining 2% cholesterol and limited in protein, were fed by stomach tube to all rats in three equal feedings daily. Thus any dietary variables were eliminated from this phase of the experiment. The animals were deliberately overfed in an attempt to induce obesity and hyperlipemia. The tube-feeding was discontinued after six weeks because this was considered to be the optimum point of tolerance for such a procedure. The injections were administered daily throughout the entire experiment. At the end of the tube-feeding phase of the experiment, a number of rats from each group were killed for histopathological studies. The remaining animals were fed a dry diet ad libitum for the duration of the experiment. At the end of the 20th week, all survivors were killed. Each rat was bled at 2, 4, 6, 12, 18, and 20 weeks for serum lipid determinations.

Animals and Care

Sixty female and seventy male young adult albino rats of the Sprague-Dawley strain, 4 to 6 months of age, were used to form the experimental groups. The average weight of the females was 240 gm. (212-268 gm.) and that of the males was 387 gm. (351-447 gm.). All the rats had been maintained on Purina Laboratory Chow prior to this experiment. They were kept in a thermostatically controlled room (76 F), in large wire-bottomed cages with a maximum of 10 rats per cage. To ensure constant and adequate dietary intake, measured amounts of the synthetic liquid diet were fed by stomach tube to all rats at regular six-hour intervals three times daily, with use of the technique of Shay and Gruenstein,[37] aided by an apparatus patterned after that designed by Talalay.[35] In keeping with the differences in their body weights, the female animals received 9 cc. and the male animals received 14 cc. at each feeding. During the final 14 weeks of the experiment the rats were fed weighed portions of a solid synthetic diet each afternoon, the uneaten ration being weighed and discarded the following day. Diet consumption records were kept throughout this phase of the experiment. The animals were weighed two or three times weekly. The compositions of the rations used are given in Table 2. Fresh diets were prepared weekly and were stored in the dark at 5 C, in covered glass containers. The liquid diet was similar to a ration used previously in this laboratory in experiments studying the effect of diet on the serum cholesterol-lipid phosphorus ratio.[40] The dry diet was similar to one which has previously been shown to be atherogenic for rats.[26] Both of the rations used in this experiment contained a higher level of cholesterol than had been used previously in this laboratory for chronic feeding experiments.

Table 2.—*Diet Composition*

Ingredients and Sources	Units	Liquid	Dry
Vitamin test casein, Nutritional Biochemicals, Inc.	Gm.	0.0	10.0
Dehydrated egg (defatted)*	Gm.	2.5	0.0
Lard, Swift's Clover Leaf	Gm.	0.0	30.0
Corn oil, Mazola	Gm.	25.0	0.0
Dextrin (white), Steinhall, RH4	Gm.	7.75	47.5
Sucrose	Gm.	3.75	0.0
Alphacel, Nutritional Biochemicals, Inc.	Gm.	4.0	3.5
Salt mixture†	Gm.	2.0	6.0
Choline chloride, Nutritional Biochemicals, Inc.	Gm.	0.5	1.0
Animal cholesterol U. S. P., The Wilson Laboratories	Gm.	2.0	2.0
Water	Cc.	Q.S.	0.0
Total	....	100.0 Cc.	100.0 Gm.
Calories per cubic centimeter (tube-fed) or per gram (ad lib.)	....	2.8	5.0
Vitamins:			
Thiamine HCl, Merck & Co., Inc.	Mg.	0.5	0.7
Riboflavin, Merck & Co., Inc.	Mg.	1.0	1.4
Nicotinic acid, Merck & Co., Inc.	Mg.	1.5	2.1
Pyridoxine HCl, Merck & Co., Inc.	Mg.	0.5	0.7
Ca pantothenate, Merck & Co., Inc.	Mg.	1.5	2.1
Menadione, Nutritional Biochemicals, Inc.	Mg.	4.0	5.6
Percomorph liver oil, Mead, Johnson & Company	Gtt.	0.2	0.5

* The dehydrated eggs were defatted by three extractions with a mixture of acetone, alcohol, and ether.
† Hawk and Oser's modification of the Osborne and Mendel salt mixture.[39]

Castrations

Bilateral orchidectomies were performed through a small incision in the tip of the scrotum after ligation of the spermatic cords. Bilateral ovariectomies were accomplished through dorsal paramedial incisions at the level of the lower poles of the kidneys. Ligatures passed around the distal ends of the uterine horns provided adequate hemostasis. The above procedures were performed with the animal under Cyclopal (5-allyl-5-[2-cyclopenten-1-yl]-barbituric acid) anesthesia, using clean but not sterile technique. Blood loss was minimal, and there were no surgical complications. All wounds were closed with silk suture for muscle layers and wound clips for skin. The clips were removed at eight days.

Hormone Preparations

Each rat in Groups V and VI (Table 1) received daily subcutaneous injections of 0.2 cc. of Synandrol,‡ which contained 5.0 mg. of testosterone propionate in sesame oil. Each rat in Groups VII, VIII, and IX (Table 1) received daily subcutaneous injections of 0.2 cc. of Progynon benzoate,§ which contained 0.333 mg. of estradiol benzoate in sesame oil. All of the other animals were injected with 0.2

‡ The Synandrol was generously donated by Pfizer Laboratories, Brooklyn.

cc. of plain sesame oil § daily to obviate any effects of the vehicle.

Serum Lipid Determinations

Twelve-hour-fasting blood samples were obtained from the lateral tail vein of each rat using the method of Cannon and co-workers.[41] The serum was separated from the clot by centrifugation. Serum cholesterol and lipid phosphorus values were determined on a small portion of each sample by standard micromethods.‖

Histopathological Technique

All animals were autopsied when they died or when they were killed during the experiment. The rats to be killed were exsanguinated from the heart, under ether anesthesia. Most of the organs were examined grossly and tissue samples of the heart (transverse and frontal blocks), entire aorta, lung, superior mediastinum, thyroid, liver, spleen, pancreas, adrenals, kidneys, gonads, and secondary sex organs were fixed in neutral formalin-saline. Duplicate blocks were taken of the aorta (both ends), kidney, heart, and, in some instances, liver for preparation of frozen sections, which were stained for neutral fat with Sudan IV. The other tissue blocks were embedded in paraffin, cut at 6μ, and stained with eosin-azure.

§ The Progynon-B and plain sesame oil were generously donated by the Shering Corporation, Bloomfield, N. J.

‖ References 42 and 43.

RESULTS

Body Weights and Food Consumption

Each of the experimental groups showed similar increases in body weights during the first six weeks while they were being tube-fed (Chart 1). Immediately after cessation of the tube-feeding the rats began eating ad libitum, and their weights dropped precipitously during the next four to six weeks. For the duration of the experiment, most of the females remained at or slightly above the levels they had reached before the experi-

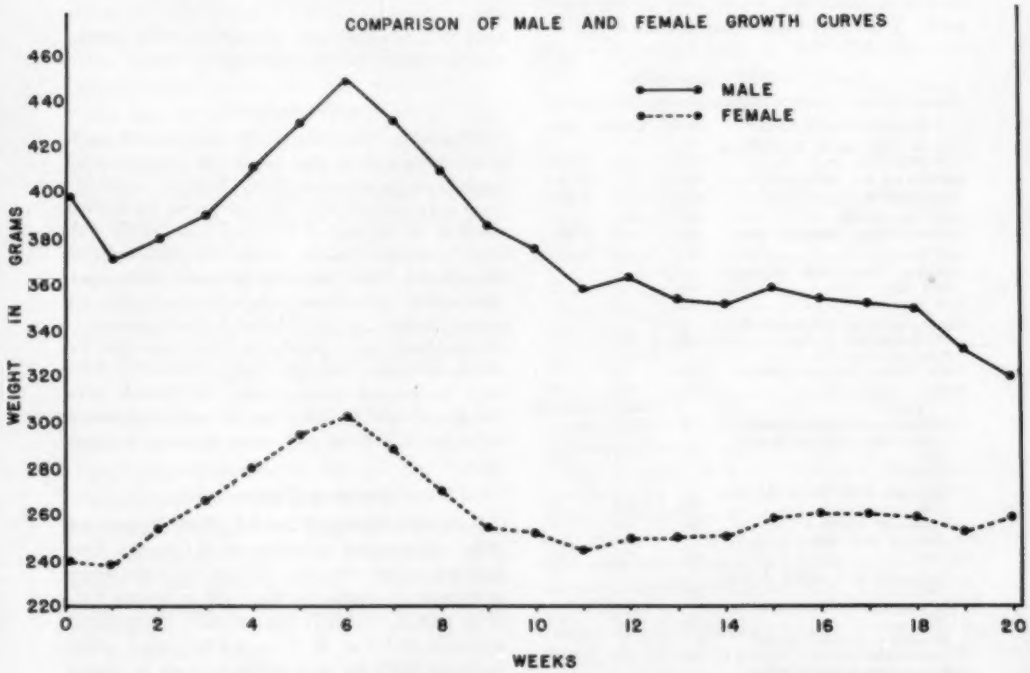

Chart 1.—The upper curve represents the mean weekly weight changes of the male rats (Groups II, IV, VI, and IX). The lower curve represents the mean weekly weight changes of the female rats (Groups I, III, V, and VII). Each successive point on both curves represents a decreasing number of animals due to deaths. Part of the weight losses may be accounted for by a moderate to severe pneumonitis which affected the entire rat colony.

ment started. Many of the males, on the other hand, continued to lose weight gradually and by the end of the experiment had fallen to levels somewhat below their initial weights. The mean weight changes for the males and females are shown graphically in Chart 1. All groups showed similar changes in weight during the experimental period. This sex difference in weight loss is probably related to the decreased level of daily diet consumption, which was comparable in most of the rats irrespective of age, sex, or body size. At such low levels, the food intake was sufficient to maintain the 250 gm. female animals, but was probably inadequate for the male animals, whose average weight was 400 gm.

Serum Lipid Determinations

The mean values for the serum levels of cholesterol, lipid phosphorus, and the cholesterol-lipid phosphorus ratio of each group are tabulated in Table 3. The serum lipid changes in response to estradiol, castration, and testosterone have been summarized graphically in Chart 2. Here, the rats with each type of treatment were compared with those without. It is evident that estradiol had a more appreciable effect on the serum lipid levels than either castration or testosterone. At six weeks, the rats with estradiol treatment exhibited cholesterol and lipid phosphorus levels significantly higher than those in the animals without such treatment. At 18 weeks, the serum cholesterol values in the rats with estrogen treatment and in those without tended to rise to approximately the same high level, but the lipid phosphorus values in the estrogen-treated rats continued to be much higher than in those not receiving estradiol. Thus, the cholesterol-lipid phosphorus ratios rose

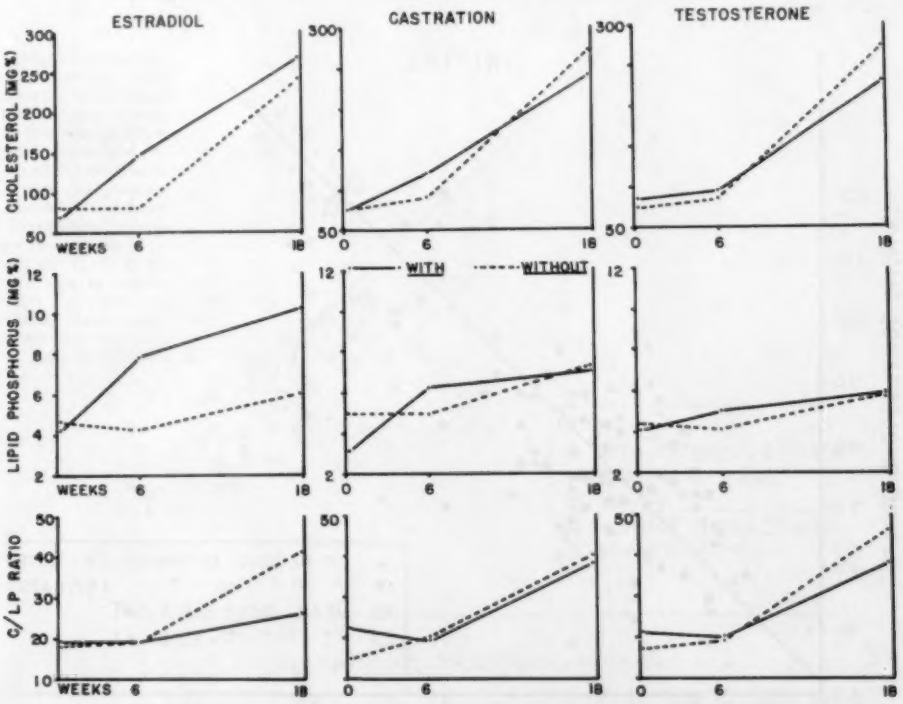

Chart 2.—Comparison of the influence of estradiol, castration, and testosterone on serum lipid concentrations. The controls, i. e., "without," demonstrate the effects of diet alone.

TABLE 3.—*Averaged Serum Lipid Data for Each Group of Rats*

Group	Sex	Experimental Procedure	Serum Lipids, Mg. per Cent	Weeks 0	6	12	18
I	F	.....	Cholesterol	85*	91	219	305
			Lipid phosphorus	4.9*	3.8*	4.0	6.3
			C:LP†	17.3*	21.7	55.8	49.5
II	M	.....	Cholesterol	74*	70	107	241
			Lipid phosphorus	5.0*	3.8	3.3	5.8
			C:LP	14.8*	18.9	32.4	41.7
III	F	Castration	Cholesterol	74	98	263	336
			Lipid phosphorus	3.8	4.8	6.3	6.0
			C:LP	19.5	20.0	42.7	56.9
IV	M	Castration	Cholesterol	76	94	189	207
			Lipid phosphorus	3.5	5.5	6.1	6.0
			C:LP	21.8	17.4	32.4	36.6
V	F	Castration and testosterone	Cholesterol	92	92	298	....‡
			Lipid phosphorus	4.6	5.3	6.2	....
			C:LP	20	17.5	46.6	....
VI	M	Testosterone	Cholesterol	77	101	130	229
			Lipid phosphorus	3.6	4.8	5.9	5.7
			C:LP	21.5	21.2	21.8	37.5
VII	F	Estradiol	Cholesterol	75	151	226	288
			Lipid phosphorus	4.5	8.3	11.2	11.0
			C:LP	17.5	17.9	29.3	25.9
VIII	M	Castration and estradiol	Cholesterol	78	146	247	217
			Lipid phosphorus	3.3	7.9	10.2	9.2
			C:LP	24.1	18.3	24.4	24.0
IX	M	Estradiol	Cholesterol	68*	129	218	226
			Lipid phosphorus	4.8*	7.3	5.4	10.3
			C:LP	14.2*	19.2	41.7	28.8

* Determinations done on pooled sera samples.
† C:LP = Serum cholesterol:lipid phosphorus ratio.
‡ Not determined.

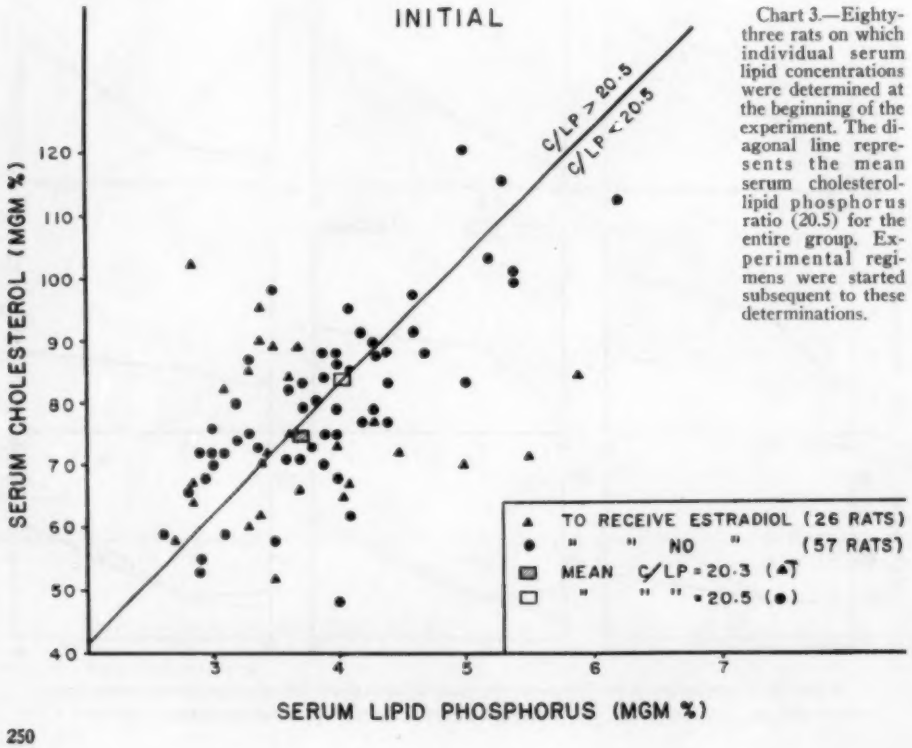

Chart 3.—Eighty-three rats on which individual serum lipid concentrations were determined at the beginning of the experiment. The diagonal line represents the mean serum cholesterol-lipid phosphorus ratio (20.5) for the entire group. Experimental regimens were started subsequent to these determinations.

to very high levels (mean value, 41.7) in the untreated groups, whereas the rats receiving estradiol had significantly lower ratios (mean value, 26.3) at the end of the experiment. This latter value approaches the mean cholesterol-lipid phosphorus ratio (20.5) which had been determined for the entire rat population at the beginning of the experiment (Chart 3).

The specific effect of estradiol upon serum phospholipid concentration in individual rats is illustrated graphically in Charts 4 and 5. Each rat on which serum lipid determinations were obtained is represented, and those receiving estradiol are identified. It may readily be seen that although the estradiol-treated rats were widely distributed with respect to cholesterol levels, they were sharply demarcated from the rest with respect to lipid phosphorus values. This delineation was even more striking at 18 weeks than it was at 6.

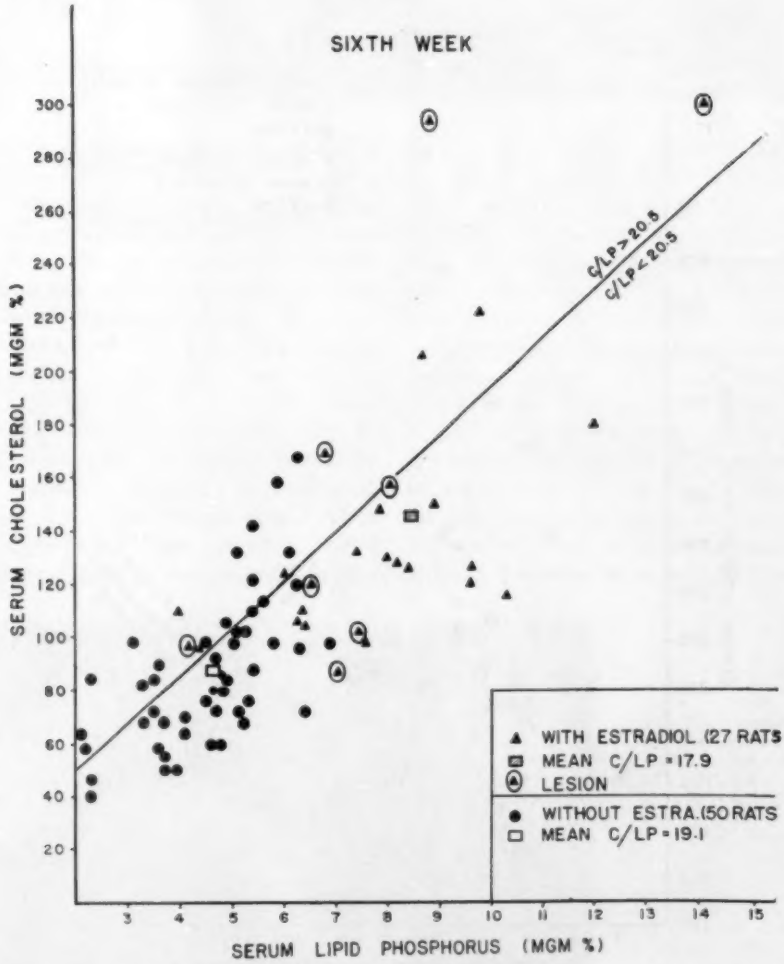

Chart 4.—Seventy-seven rats on which serum lipid concentrations were determined at six weeks. The diagonal line represents the mean serum cholesterol-lipid phosphorus ratio determined for normal untreated rats, eating a stock diet (compare Chart 3).

The trends of the serum lipid values in the castrated rats and in those with testosterone treatment, on the other hand, did not vary significantly from those in the animals without such treatment.

Histopathologic Observations

The most remarkable microscopic finding was the presence of Sudanophilic lipid in the walls of the coronary arteries. These lesions were of the same variety as those produced by Wissler [26] with dietary imbalance for 26 weeks or longer. Two types of changes were described by Wissler: If the lipid deposition was accompanied by intimal proliferation and/or by definite degeneration of the arterial wall, he called it atheromatous; otherwise, it was called lipomatous, but he suggested that these two types of lesions probably represented varying degrees of the same process. In the present experiment 47% of the rats with coronary lesions at 20 weeks showed moderate to severe atheromatous changes. It is significant that none

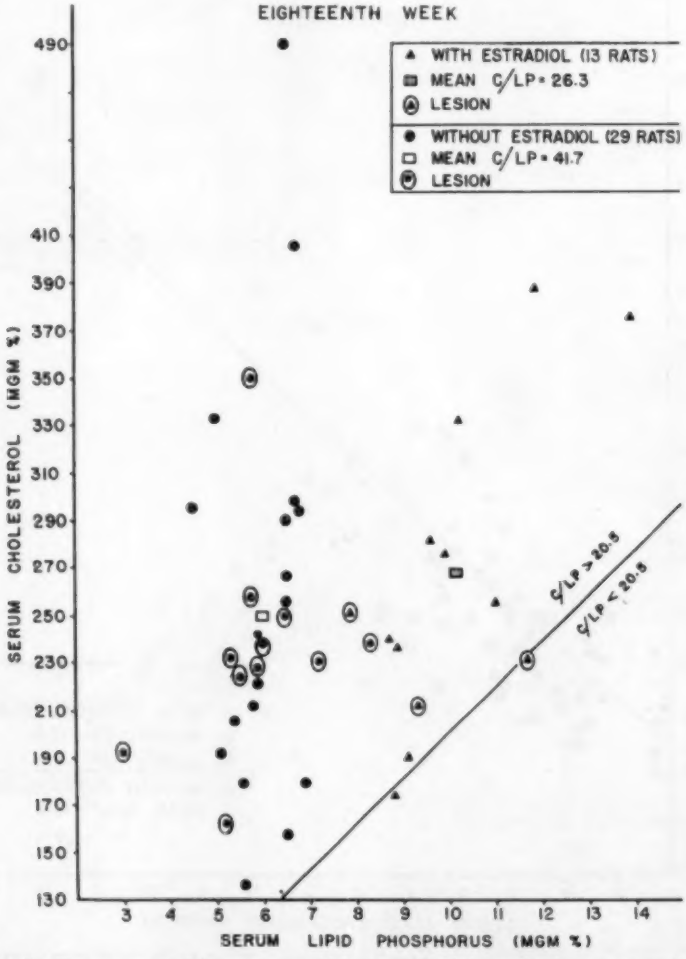

Chart 5.—Forty-two surviving rats on which serum lipid determinations were performed at 18 weeks. The diagonal line represents the base-line serum cholesterol-lipid phosphorus ratio (compare Chart 3).

Fig. 1.—Minimal lipomatous lesion in a coronary artery of a male rat treated with testosterone propionate, killed at 136 days. The black areas in the arterial wall are small- and medium-sized fat droplets. Frozen section, Sudan IV-hematoxylin; reduced about ⅓ from mag. × 230.

of the lesions appearing in estrogen-treated rats at the end of the experiment could be called atheromatous, despite the fact that they had presumably developed earlier than those in the other groups. All of the coronary lesions in rats surviving less than 20 weeks showed only lipomatous changes. The lipid-containing lesions were found in small- and medium-sized coronary branches only, the larger coronary arteries being completely unaffected. The minimal lesions (Fig. 1) consisted of focal collections of Sudanophilic droplets in the medial layers of small arteries. The media did not appear otherwise altered, and there were no intimal changes or endothelial involvement. The most advanced lesions (Figs. 2 and 3) consisted of large coalescent fat droplets in the intima, which formed a plaque encroaching upon the lumen and obscuring the endothelial surface. These lesions extended down into the media, where smaller discrete fat droplets were present. Most of the lesions observed in this experiment ranged between these two degrees of severity. Although there were significant variations in the incidence of lesions from group to group, no real group differences with respect to quality or severity of lesions could be established. There was no difference in the quality or intensity of lesions observed in the estrogen-treated rats at 6 and 20 weeks (Figs. 4 and 5). Intimal

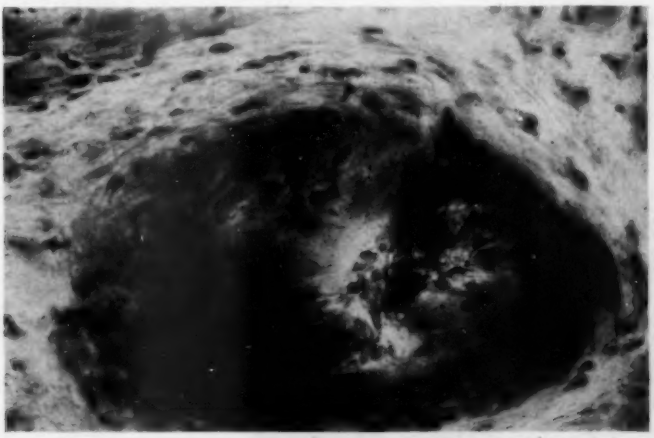

Fig. 2.—Advanced atheromatous degeneration in a medium-sized coronary artery of a castrated male rat, killed at 135 days. There are large, coalescent fat globules throughout the media, and the lumen is narrowed by intimal proliferation. Frozen section, Sudan IV-hematoxylin; reduced about ⅓ from mag. × 590.

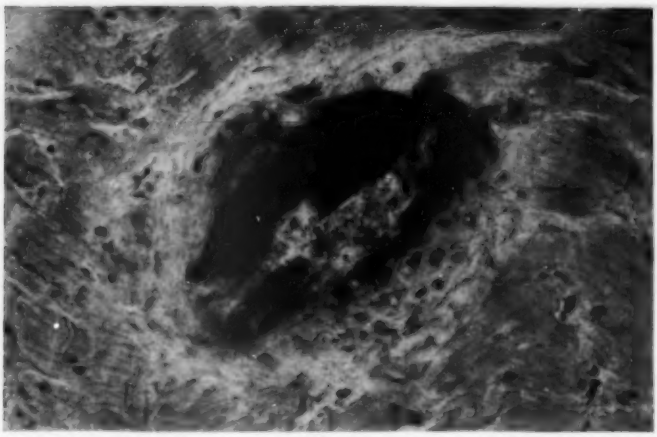

Fig. 3.—A small coronary artery of a castrated male rat, killed at 136 days, exhibiting similar changes to those described in Figure 2. Frozen section, Sudan IV-hematoxylin; reduced about ⅓ from mag. × 560.

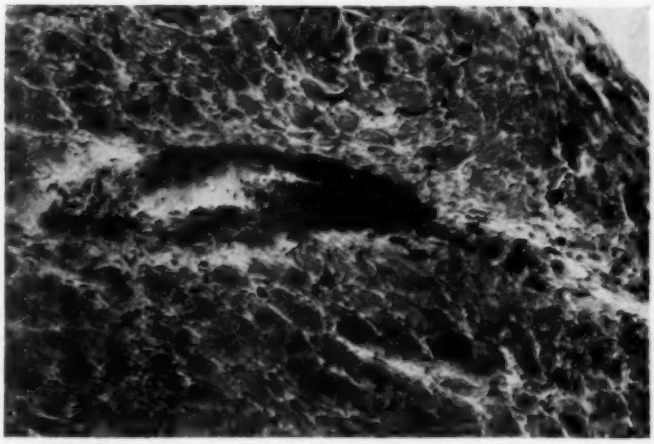

Fig. 4.—Moderate-sized coronary artery of a castrated male rate receiving estradiol benzoate, killed at 44 days. Frozen section, Sudan IV-hematoxylin; reduced about ⅛ from mag. × 230.

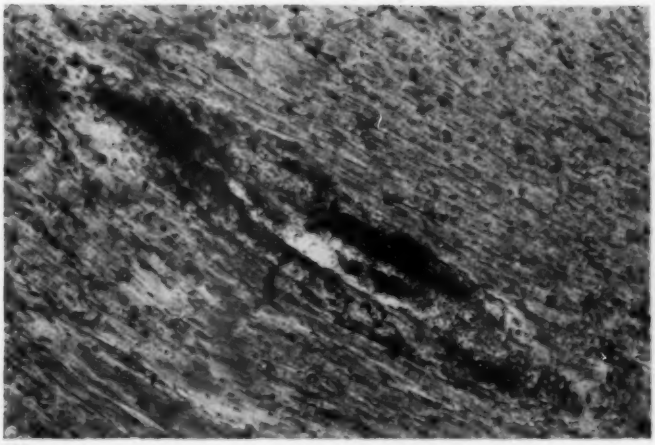

Fig. 5.—Moderate-sized coronary artery of a castrated male rat receiving estradiol benzoate, killed at 136 days. Frozen section, Sudan IV-hematoxylin; reduced about ⅛ from mag. × 230.

plaques are well demonstrated in Figure 6. The only vascular changes noted on the eosin-azure-stained heart sections consisted of signet-ring cells in the coronary arteries. These cells had the appearance of lipocytes, but they occurred individually in the media and, in some cases, in the intima of the small coronary arteries. They were round with clear, pale-staining, vacuolated cytoplasm and contained a small basophilic nucleus flattened against the periphery. These signet-ring cells occurred in all groups in varying numbers, and their incidence could not be positively correlated with castration, estrogen, or testosterone treatment. It is noteworthy, however, that of the 76 rats in which these cells were found 50 were male and only 26 were female. The occurrence of these cells could not be related to the length of time the animals had been receiving the experimental diet. They were found in rats which had died as early as 11 days after the experiment began as well as in rats surviving 20 weeks.

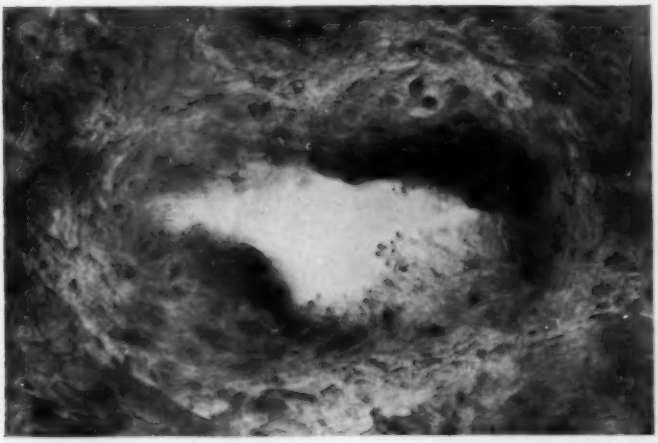

Fig. 6.—Focal intimal thickening and medial degeneration in a small coronary artery of a castrated male rat, killed at 135 days (in the same rat as the artery shown in Fig. 2). Frozen section, Sudan IV-hematoxylin; reduced about ⅓ from mag. × 520.

No inflammatory changes were noted in the myocardium of any of the animals, and there was no evidence of coronary thrombosis or infarction. No gross or microscopic changes were observed in any of the aortic sections despite the degree of coronary involvement.

Arteritis was not a prominent finding in either the heart or kidney sections. No lipomatous or atheromatous changes could be demonstrated in the renal vessels with the Sudan IV stain. The only finding of note in the kidneys was foci of dilated tubules containing a hyaline eosinophilic precipitate. This change was demonstrable in animals from each group of male rats, regardless of their treatment, but was conspicuously absent in the female rats, whether intact or castrated.

Most of the livers from each group showed a marked degree of fatty change, but there was no evidence of dietary cirrhosis. Presumably these animals had not been subjected to the high-fat diet for a sufficient length of time, or the choline content of the diet was high enough to prevent the development of this lesion. However, in all groups there were examples of the "massive acute necrosis of the liver" which Himsworth and Glynn have described.[44] This lesion is apparently related to a deficiency of cystine and/or methionine and alpha-tocopherol.[45] It is probably significant that this hepatic necrosis was found almost exclusively among rats which had become sick and died before the experiment ended. There was no correlation between the hepatic necrosis and the incidence of lipid-containing coronary lesions.

There were two other noteworthy pathologic processes, but they are both common to the albino rat and probably had no bear-

ing on the experiment whatsoever. Most of the animals in each group had a mild to moderate chronic bronchitis. In many instances this was accompanied by suppurative bronchiectasis and focal chronic pneumonitis. Many rats also showed a chronic suppurative otitis media at autopsy. In some instances there were associated clinical signs suggesting labyrinthian disease.

Factors Influencing Coronary Lipid Deposition

The incidence of coronary artery lipid-containing lesions in the various groups is summarized in Table 4. For purposes of tabulation, all rats showing lipid deposition in one or more coronary arteries, regardless of severity, were considered positive for lesions. Since the earliest such change was noted at 32 days, only animals surviving for this period of time or longer were considered in the tabulation. Each group was divided into two parts to demonstrate the importance of the time factor in this experiment. Part I includes 58 rats which died or were killed between 32 and 54 days, and Part II, 50 rats which were autopsied between the 90th and 143d day. It is to be noted that during the first 54 days of this experiment coronary lesions were observed only in those rats receiving estradiol (Table 4, Part I). During the latter time period (Table 4, Part II), the incidence of lesions in the estrogen-treated groups declined sharply, and coronary lipid deposition was observed to occur predominantly among the animals without estrogen treatment. Of the 13 estrogen-treated survivors at the end of 143 days, only 3 showed lesions, indicating a marked decrease in incidence from that noted in the earlier part of the experiment. Correlated with this, the lipid phosphorus values continued to increase in the estrogen-treated rats (Table 3; Chart 2). At 20 weeks the cholesterol values were comparably elevated in all animals, whether or not they were receiving estradiol. Consequently, much higher serum cholesterol-lipid phosphorus ratios were observed among those animals without estro-

Table 4.—*Incidence of Lipid-Containing Coronary Lesions in Each Group During Early and Late Phases of the Experiment*

			Part I, 32-54 Days			Part II, 90-143 Days		
Group	Sex	Experimental Procedure	No. of Rats	No. of Rats with Lesions	Incidence of Lesions	No. of Rats	No. of Rats with Lesions	Incidence of Lesions
I	F	.....	5	0	0%	5	1	20%
II	M	.....	4	0	0%	10	5	50%
III	F	Castration	9	0	0%	4	0	0%
IV	M	Castration	4	0	0%	10	6	60%
V	F	Castration and testosterone	4	0	0%	3	0	0%
VI	M	Testosterone	10	0	0%	5	1	20%
VII	F	Estradiol	9	4	44%	3	1	33%
VIII	M	Castration and estradiol	8	4	50%	5	2	40%
IX	M	Estradiol	5	3	60%	5	0	0%
Total males			31	7	23%	35	14	40%
Total females			27	4	15%	15	2	13%

Table 5.—*Influence of Estradiol Benzoate, Testosterone Propionate, and Castration on the Incidence of Lipid-Containing Coronary Lesions and the Cholesterol: Lipid Phosphorus Ratio in the Rat During Early and Late Phases of the Experiment*

	No. of Rats	No. of Rats with Lesions	Incidence of Lesions	C:LP
Part I, 32-54 days				
Estradiol	22	11	50%	18.5*
Untreated	13	0	0%	19.8*
Testosterone	14	0	0%	19.6*
Untreated	13	0	0%	19.8*
Castration‡	21	4	19%	18.4*
Gonads intact‡	14	3	21%	19.8*
Part II, 90-143 days				
Estradiol	13	3	23%	26.3†
Untreated	25	12	48%	41.1†
Testosterone	8	1	13%	36.4†
Untreated	14	5	36%	46.4†
Castration‡	19	8	42%	37.5†
Gonads intact‡	20	6	30%	39.9†

* Mean serum cholesterol:lipid phosphorus ratio at 6 weeks.

† Mean serum cholesterol:lipid phosphorus ratio at 18 weeks.

‡ Includes animals treated with estradiol.

gen treatment, averaging 41.7 as compared with 26.3 in the estrogen-treated rats (Chart 5). This association of elevated serum cholesterol-lipid phosphorus ratios with an increased incidence of lesions is in accord with the results of many previous studies in the rat and other species.¶

The differential incidence of lipid-containing coronary lesions at the end of the experiment with respect to sex is also summarized at the end of Table 4. These data suggest that the adult male rat is more susceptible to experimental coronary lipid deposition than is the adult female rat.

¶ References 23-28.

The influence of each of the three experimental variables (estradiol, testosterone, and castration) on the incidence of lesions is summarized in Table 5, and is shown graphically in Charts 6 and 7. It is evident that during the early phase of the experiment the occurrence of coronary lesions was definitely associated with estradiol administration. On the other hand, in the later phase, the incidence of coronary lesions among the untreated rats was more than twice as great as in the estradiol-treated groups. Testosterone had no such atherogenic action in the early phase of the experiment, but later it was observed that the incidence of lesions

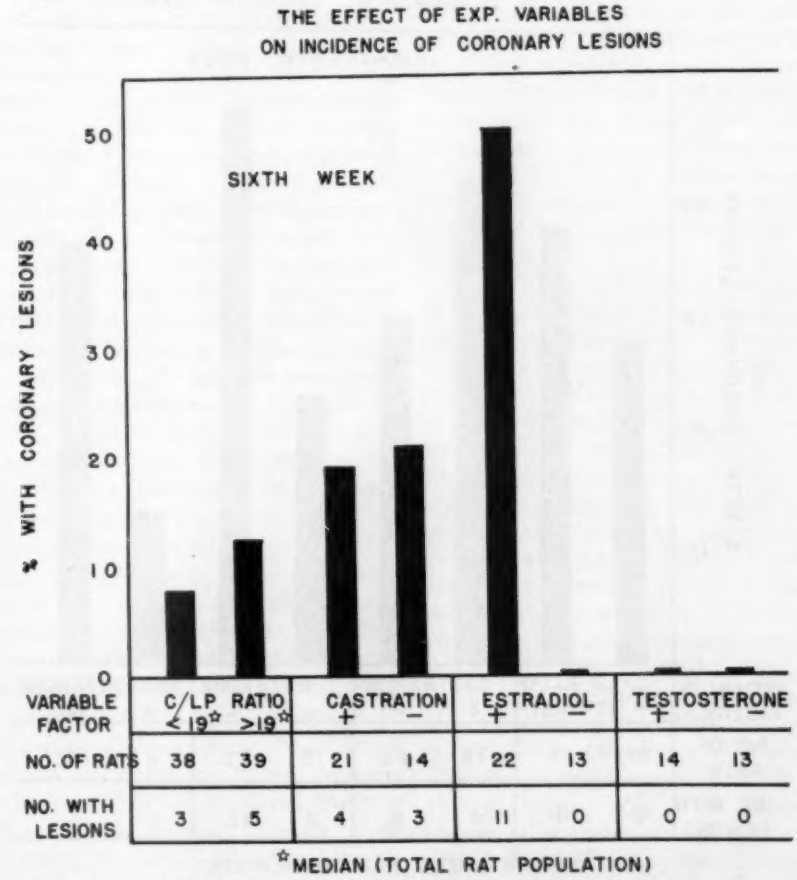

Chart 6.—The influence of various hormonal factors on the incidence of lipid-containing coronary lesions in the rat at six weeks by comparison with controls. The association of incidence of lesions with the serum cholesterol-lipid phosphorus ratios is also demonstrated.

in testosterone-treated animals was only one-third as high as in a comparable group of untreated controls. Thus, the possibility that long-term testosterone treatment may be antiatherogenic must be considered. Castration, however, had no effect on the accumulation of lipid in the coronary arteries during any phase of the experiment.

The coronary lesions appearing at 90 to 143 days were definitely associated with elevated cholesterol-lipid phosphorus ratios (Table 5; Chart 5) except in those animals receiving estradiol. Among the estrogen-treated rats during this latter period, the incidence of coronary lipid lesions had definitely decreased, and the cholesterol-lipid phosphorus ratio was significantly reduced.

During the early phase of the experiment, coronary lesions occurred among the estrogen-treated rats in substantial numbers despite ratios of less than 20. However, it is notable that those lesions which occurred in the estrogen-treated animals at six weeks were associated with the lower lipid phosphorus values, except for two whose serum cholesterol values were exceedingly high (Chart 4).

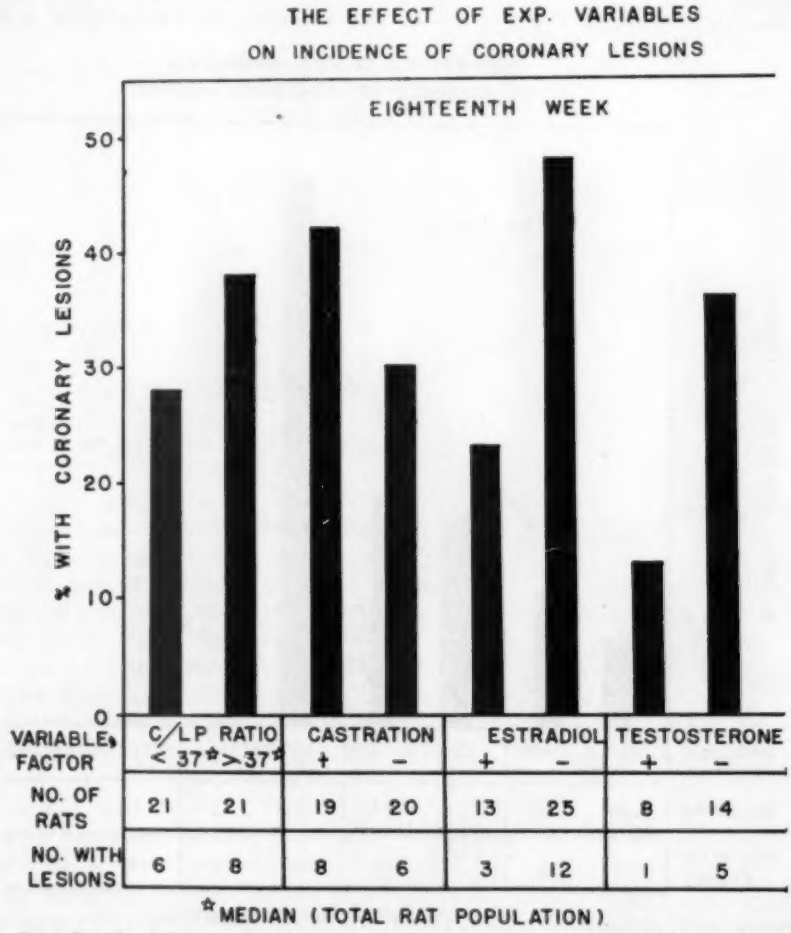

Chart 7.—The influence of various hormonal factors on the incidence of lipid-containing coronary lesions in the rat at 18 weeks by comparison with controls. The association of incidence of lesions with the serum cholesterol-lipid phosphorus ratios is also demonstrated.

COMMENT

The results of this study may help to explain some of the seemingly conflicting data found in the experimental literature regarding the influences of estrogens on coronary atherosclerosis. The main points at variance are estrogen induction [12] and estrogen inhibition [13] of coronary atheromatosis in the chick. This apparent paradox is clearly demonstrated in the rat in this experiment. While it appears probable that the diet was a necessary predisposing factor, there can be little doubt that exogenous estrogens induced lipid-containing lesions in the coronary arteries during the first eight weeks of the experiment. During that period lesions occurred only in groups receiving estradiol. In the later phase of the experiment this trend was reversed, with many lesions being observed in the groups without estrogen treatment, and there was a marked reduction of incidence of lesions in the estradiol-injected rats (Table 5). Thus, if some of the rats had not been killed early in the experiment, the acute effects of estradiol would have been masked, and it might easily have been inferred from the final results that estrogen injections had no effect other than inhibition of coronary atheromatous changes. But since it is evident that the estrogen-treated rats exhibited a marked decrease in incidence and severity of lesions as the experiment progressed, it seems more valid to describe this as a regressive phenomenon. It is evident from the individual group data (Table 4) that this apparent regression is more marked among the intact male animals receiving estradiol than in either the castrated male animals or the intact female animals similarly treated. The incidence of lesions in the former group was 60% in the early phase of the experiment and fell to 0% during the final period, whereas among the castrated male and the intact female rats receiving estradiol there was no significant change in incidence during the final 12 weeks of the experiment. Therefore this regressive response to chronic estrogen administration appeared to be partially dependent on the presence of the testis, despite the fact that this organ showed histological evidence of atrophy.

Observations on the severity of the lesions also support the conclusion that estradiol has a regressive effect or a limiting effect upon atheromatous change in the coronary arteries of the rat. The lesions in the animals not receiving estradiol were severer at the end of the experiment than those in the estrogen-treated rats, despite the fact that the estrogen-enhanced lesions apparently had developed earlier.

It is difficult to account for this apparent dual effect of estradiol on the male rat. It is possible that although the primary action is to enhance development of lipid-containing coronary lesions, secondary mechanisms may be elicited later which tend to reverse the original effect. It might also be argued that the estradiol acts synergistically with the tube-fed diet to augment development of coronary lesions, and antagonistically in conjunction with the ad libitum diet, but this seems unlikely since the two rations were basically similar.

In this, as in previous studies, the lack of correlation between hypercholesteremia, per se, and the presence of lipid-containing lesions in the coronary arteries is well demonstrated. As indicated in Table 3, the level of serum cholesterol at the end of the experiment was about equally elevated in the rats treated with estradiol as in the corresponding control animals. Yet, the incidence of coronary fatty lesions was more than twice as great in the control rats. Moreover, a still higher incidence of lesions was observed earlier in the experiment among the estradiol-treated rats despite lower serum cholesterol levels.

The serum cholesterol-lipid phosphorus ratio, nevertheless, was definitely associated with the incidence and severity of atheromatous changes in some groups. At the end of the experiment, when their lesions were regressing, the mean serum cholesterol-lipid phosphorus ratio of the estradiol-treated rats was 26, whereas the untreated controls, which showed a contrastingly high incidence of

atheromatous coronary lesions, had ratios which averaged 42. Some were as high as 68. This latter observation, relating the presence of lipid-containing coronary lesions to elevated serum cholesterol-lipid phosphorus ratios, is in accord with a number of independent observations in many species, including the rat.# The corollary observation, relating the lower incidence of lesions in chronically estrogen-treated rats to decreased serum cholesterol-lipid phosphorus ratios, confirms, in the rat, previous observations relating estrogen protection to an elevated serum phospholipid concentration in the cockerel.[13] However, in the early part of this experiment, hyperphospholipemia exerted a less definite effect on the development of lesions in the estrogen-treated animals, despite its suppressive effect on the ratio.

If the terminal cholesterol-lipid phosphorus ratios, which were determined on 90 rats, had been considered collectively, irrespective of the length of time each animal had been on the experimental regimen, the conclusions would be different. The results then would have shown that 15 of the 45 animals with ratios above 24 exhibited lesions, while of the 45 rats with ratios below 24, lesions appeared in 11. This might have been interpreted as showing no relationship between the presence of lipid in the walls of coronary arteries and alterations in the serum lipid pattern. That interpretation obviously cannot be supported when the data for the two time periods are considered separately.

There are no data in this experiment to support the contention that testosterone has any deleterious action on the coronary arteries, or that its influence on coronary atherosclerosis is opposed to that of estrogens. In fact, there is suggestion that a decreased incidence of lipid-containing coronary lesions is associated with the chronic administration of testosterone, similar to that observed with chronic estrogen-treatment. However, owing to the small number of animals involved in that group, no definite conclusions may be reached at this time concerning the effects of the male hormone. In this connection there has been a recent report [26] concerning the regression of aortic atheromata in the rabbit subsequent to testosterone administration. In the present experiment castration appeared to be entirely without effect on the coronary arteries and serum lipids of male and female rats, regardless of whether or not they were receiving exogenous hormones.

It is not suggested that the pathologic responses of the rat are identical with those of any other species, but it is noteworthy that there is a sex difference with regard to susceptibility of the rat to development of fatty lesions in the coronary arteries similar to that of man * and the chicken.[47] Of 35 male rats surviving 90 days or longer, lipid-containing coronary lesions were found in 14 (incidence, 40%), whereas in a similar group of 15 females, only 2 of the rats showed lesions (incidence, 13%). Thus, the ratio of males to females with regard to susceptibility to coronary fatty lesions is 3:1 in this series of rats. This ratio is consistent with the rarity of coronary atherosclerosis among female human beings during the reproductive years.[48] The hyperphospholipemic response to chronic administration of estradiol, the association of coronary fatty lesions with an altered serum cholesterol-lipid phosphorus ratio, and the dichotomy between the severity of aortic and coronary lesions have all been reported in man and were all observed in the rat in this experiment. They stand as further points in favor of a similarity of those two species with respect to coronary atherogenesis.

The results of this experiment also serve to confirm the report of Wissler and coworkers [26] concerning the production of atheromatous coronary lesions in rats with use of a cholesterol-containing synthetic ration with high levels of fat and choline. Middle-aged obese rats were employed in that experiment, and the importance of age and preexisting obesity as contributory factors was discussed. No lesions were ob-

References 23-28.

* References 5 and 46.

served by Wissler until 26 weeks of dietary treatment had elapsed. It is therefore noteworthy that similar lesions were obtained in this experiment, but with a shorter experimental period and much younger rats than those employed previously. The most significant differences between the dietary regimen in these two experiments was the initial six-week period of tube-feeding and the higher cholesterol level employed. One or both of these factors probably account for the earlier occurrence of coronary fatty lesions in the present study.

In neither of these experiments did maintenance of obesity appear to be a necessary predisposing factor to the development of coronary fatty lesions, but, in both instances, initially well-filled fat depots may have been an important prerequisite to the development of lesions. In addition, it is possible that the early high-caloric regimen induced by tube-feeding, which resulted in overfilling of fat depots, may have hastened the rate at which lesions developed in this study. In the final weeks of the experiment both the incidence and severity of lesions were highest in groups showing the greatest weight loss, suggesting a possible direct association with increased depot fat mobilization. Whether this association of coronary involvement with weight loss is correlated with the elevated serum lipid concentrations, altered serum cholesterol-lipid phosphorus ratios, or a change in the character of the circulating lipids, requires more investigation.

The rat has long been described as an unsuitable animal for the study of experimental atherosclerosis,† presumably because of its unusual resistance to the disease. But from another point of view, this resistance may be considered most desirable, since it allows for a better controlled study of the various factors involved in the evolution of the disease without the masking effect produced by the unusual susceptibility to dietary cholesterol found in the rabbit and the fowl. Recent studies by Malinow and co-workers,‡ Hartroft and co-workers,[54] Wilgram and co-workers,§ Bragdon and Mickelsen,[57] Wissler and co-workers,‖ as well as the present study, indicate that there is much to be learned regarding the natural history of atherosclerosis by investigating the nature of the rat's resistance to it. Thus, in the rat, it seems likely that atheromatous lesions resulting from any set of experimental conditions must be considered the manifestation of highly potent atherogenic factors. These factors are obviously multiple, varied, and complex, but it is probable that a study of their characteristics and relationships offers hope for a better understanding of the human disease.

† References 48-50.

‡ References 51-53.

§ References 55 and 56.

‖ References 26 and 27.

SUMMARY

One hundred thirty young adult rats of both sexes were divided into nine groups, and all were fed the same synthetic diet containing high levels of fat, cholesterol, and choline. Various groups were treated with estradiol benzoate, testosterone propionate, or castration.

Tissues were studied from rats of each group when the animals were killed at about 6 and 18 weeks or when they died. Fifty percent of the estrogen-treated rats showed accumulations of lipid in their coronary arteries during the early phase of the experiment, whereas none of the rats in other groups showed any such changes. After about 18 weeks, however, the rats receiving only dietary treatment and control injections of sesame oil showed coronary lesions in substantial numbers, whereas the incidence of lesions in the estrogen-treated groups had declined considerably. A definite correlation was observed between the presence of a terminal elevation of the serum cholesterol-lipid phosphorus ratio and the incidence of lesions.

Treatment with testosterone appeared to have no harmful effects on either the serum lipid concentrations or the coronary arteries. Castration alone was entirely without effect.

Similarities of the rat to other species with respect to atherosclerosis are noted, and the suitability of the rat as an experimental animal for the study of atherosclerosis is indicated.

REFERENCES

1. Gover, M., and Pennell, M. Y.: Statistical Studies of Heart Disease: VII. Mortality from 8 Specific Forms of Heart Disease Among White Persons, Pub. Health Rep. **65**:819, 1950.

2. Levy, R. L.; Bruenn, H. G., and Kurtz, D.: Facts on Disease of the Coronary Arteries, Based on a Survey of the Clinical and Pathologic Records of 762 Cases, Am. J. M. Sc. **187**:376, 1934.

3. Ackerman, R. F.; Dry, T. J., and Edwards, J. E.: Relationship of Various Factors to the Degree of Coronary Atherosclerosis in Women, Circulation **1**:1345, 1950.

4. Master, A. M.; Dack, S., and Jaffe, H. L.: Age, Sex and Hypertension in Myocardial Infarction Due to Coronary Occlusion, Arch. Int. Med. **64**:767, 1939.

5. Underdahl, L. O., and Smith, H. L.: Coronary Artery Disease in Women Under the Age of 40, Proc. Staff Meet., Mayo Clin. **22**:479, 1947.

6. Wuest, J. H.; Dry, T. J., and Edwards, J. E.: Degree of Coronary Atherosclerosis in Bilaterally Oophorectomized Women, Circulation **7**:801, 1953.

7. Rivin, A. U., and Dimitroff, S. P.: Incidence and Severity of Atherosclerosis in Estrogen-Treated Males, and in Females with a Hypoestrogenic or a Hyperestrogenic State, Circulation **9**:533, 1954.

8. Lindsay, S.; Lorenz, F. W.; Entenman, C., and Chaikoff, I. L.: Production of Atheromatosis in the Aorta of the Chicken by Administration of Diethylstilbestrol, Proc. Soc. Exper. Biol. & Med. **62**:315, 1946.

9. Chaikoff, I. L.; Lindsay, S.; Lorenz, F. W., and Entenman, C.: Production of Atheromatosis in the Aorta of the Bird by the Administration of Diethylstilbestrol, J. Exper. Med. **88**:373, 1948.

10. Horlick, L., and Katz, L. N.: Effect of Diethylstilbestrol on Blood Lipids and the Development of Atherosclerosis in Chickens on a Normal and Low Fat Diet, J. Lab. & Clin. Med. **33**:733, 1948.

11. Stamler, J., and Katz, L. N.: Plasma Cholesterol Phospholipid Ratio and Atherosclerosis in the Chick, Am. J. Physiol. **163**:752, 1950.

12. Lindsay, S., and Chaikoff, I. L.: Coronary Arteriosclerosis of Birds, Arch. Path. **49**:434, 1950.

13. Pick, R.; Stamler, J.; Rodbard, S., and Katz, L. N.: Inhibition of Coronary Atheromatosis in Cholesterol-Fed Chicks Receiving Estrogens, Circulation **4**:468, 1951.

14. Pick, R.; Stamler, J.; Rodbard, S., and Katz, L. N.: Estrogen-Induced Regression of Coronary Atherosclerosis in Cholesterol-Fed Chicks, Circulation **6**:460, 1952.

15. Pick, R.; Stamler, J.; Rodbard, S., and Katz, L. N.: Estrogen-Induced Regression of Coronary Atherosclerosis in Cholesterol-Fed Chicks, Circulation **6**:858, 1952.

16. Pick, R.: Estrogen-Induced Regression of Coronary Atherosclerosis in Cholesterol-Fed Chicks, Fed. Proc. **11**:122, 1952.

17. Loeb, H. G.: Effect of Estradiol-Benzoate on Serum Lipid of Rats Consuming a High Fat Diet, Proc. Soc. Exper. Biol. & Med. **49**:340, 1942.

18. Eilert, M. L.: Effect of Estrogens on the Partition of Serum Lipids in Female Patients, Metabolism **2**:137, 1953.

19. Gertler, M. M.; Hudson, P. B., and Jost, H.: Effects of Castration and Diethylstilbestrol on the Serum Lipid Pattern in Men, Geriatrics **8**:500, 1953.

20. Barr, D. P.: Some Chemical Factors in the Pathogenesis of Atherosclerosis, Circulation **8**:641, 1953.

21. Lorenz, F. W.; Chaikoff, I. L., and Entenman, C.: Endocrine Control of Lipid Metabolism in the Bird: II. Effects of Estrin on the Blood Lipids of the Immature Domestic Fowl, J. Biol. Chem. **126**:763, 1938.

22. Lindsay, S.; Nichols, C. W., Jr., and Chaikoff, I. L.: Aortic Lesions Induced in the Bird by Diethylstilbestrol Injections and Cholesterol Feeding, A. M. A. Arch. Path. **59**:173, 1955.

23. Gertler, M. M.; Garn, S. M., and Lerman, J.: Interrelationships of Serum Cholesterol, Cholesterol Esters and Phospholipids in Health and in Coronary Artery Disease, Circulation **2**:205, 1950.

24. Steiner, A.; Kendall, F. E., and Mathers, J. A. L.: Abnormal Serum Lipid Pattern in Patients with Coronary Arteriosclerosis, Circulation **5**:605, 1952.

25. Gertler, M. M., and Oppenheimer, B. S.: Total Cholesterol-Lipid Phosphorus Ratio: Its Significance in Atherosclerosis, Geriatrics **9**:157, 1954.

26. Wissler, R. W.: Production of Atheromatous Lesions in the Albino Rat, Proc. Inst. Med. Chicago **19**:79, 1952.

27. Wissler, R. W.; Eilert, M. L.; Schroeder, M. A., and Cohen, L.: Production of Lipomatous and Atheromatous Arterial Lesions in the Albino Rat, A. M. A. Arch. Path. **57**:333, 1954.

28. Davidson, J. D.; Abell, L. L., and Kendall, F. E.: Serum Lipids in Experimental Canine Arteriosclerosis, Am. Heart J. **38**:462, 1949.

29. Bruger, M.; Wright, I. S., and Wiland, J.: Experimental Atherosclerosis: V. Effect of Testosterone Propionate and Estradiol Dipropionate on the Cholesterol Content of the Blood and the Aorta in Castrate Female Rabbits, Arch. Path. **36**:612, 1943.

30. Kochakian, C. D.; MacLachlan, P. L., and McEwen, H. D.: Do Androgens Affect the Blood Lipids? J. Biol. Chem. **122**:433, 1938.

31. Zondek, B., and Marx, L.: Induction of Lipemia and Calcemia in the Cock by Means of Oestrogenic Hormone, Arch. internat. pharmacodyn. **61**:77, 1939.

32. Entenman, C.; Lorenz, F. W., and Chaikoff, I. L.: Endocrine Control of Lipid Metabolism in the Bird: III. Effects of Crystalline Sex Hormones on the Blood Lipids of the Bird, J. Biol. Chem. **134**:495, 1940.

33. Looney, J. M., and Romanoff, E. B.: Effect of Testosterone on the Serum Lipids of Normal Subjects, J. Biol. Chem. **136**:479, 1940.

34. Ludden, J. B.; Bruger, M., and Wright, I. S.: Effect of Testosterone Propionate and Estradiol Dipropionate on the Cholesterol Content of the Blood and Aorta of Rabbits, Endocrinology **28**:999, 1941.

35. Katz, L. N.; Stamler, J.; Pick, R., and Rodbard, S.: Effect of Testosterone and Chorionic Gonadotrophin on Estrogen-Induced Inhibition of Coronary Atherogenesis in Cholesterol-Fed Cockerels, Circulation **6**:474, 1952.

36. Rossi, M., and Giaquinto, M.: Il testosterone come epatoprotettore: Sinergismo d'azione del testosterone con le sostanze metilanti nelle steatosi sperimentali e nell'arteriosclerosi da carico di colesterina, Minerva ginec. **5**:295, 1953.

37. Shay, H., and Gruenstein, M.: Simple and Safe Method for the Gastric Instillation of Fluids in the Rat, J. Lab. & Clin. Med. **31**:1384, 1946.

38. Talalay, P., and Takano, G. M. V.: Apparatus for Tube-Feeding Rats, J. Lab. & Clin. Med. **40**:486, 1952.

39. Hawk, P. B., and Oser, B. L.: Modification of the Osborne-Mendel Salt Mixture, Science **74**:369, 1931.

40. Wissler, R. W.; Soules, K. H.; Schroeder, M. A., and Bradford, W. L., Jr.: Effects of Acute Dietary Imbalances on Serum Cholesterol and Lipid Phosphorus Concentrations in the Rat, Fed. Proc. **14**:423, 1955.

41. Cannon, P. R.; Humphreys, E. M.; Wissler, R. W., and Frazier, L. E.: Effects of Feeding Possible Blood Substitutes on Serum Protein Regeneration and Weight Recovery in the Hypoproteinemic Rat, J. Clin. Invest. **23**:601, 1944.

42. Abell, L. L.; Levy, B. B.; Brodie, B. B., and Kendall, F. E.: Simplified Method for the Estimation of Total Cholesterol in Serum and Demonstration of Its Specificity, J. Biol. Chem. **195**:357, 1952.

43. Fister, H. J.: Lipoid Phosphorus, Method P-37.1, Manual of Standardized Procedures for Spectrophotometric Chemistry, New York, Standard Scientific Supply Corp., 1950.

44. Glynn, L. E., and Himsworth, H. P.: Massive Acute Necrosis of the Liver: Its Significance and Experimental Production, J. Path. & Bact. **56**:297, 1944.

45. Gillman, J.; Gilbert, C.; Gillman, T., and Spence, I.: Simple Dietary Technique for the Production of Hepatic Necrosis with a Consideration of the Factors Preventing the Lesion, Am. J. Digest. Dis. **19**:201, 1952.

46. Gordon, W. H.; Bland, E. F., and White, P. D.: Coronary Artery Disease Analyzed Post Mortem: With Special Reference to the Influence of Economic Status and Sex, Am. Heart J. **17**:10, 1939.

47. Fox, H.: Some Comments on Arteriosclerosis in Wild Mammals and Birds, Bull. New York Acad. Med. **15**:748, 1939.

48. Katz, L. N., and Dauber, D. V.: Pathogenesis of Atherosclerosis, J. Mt. Sinai Hosp. **12**:382, 1945.

49. Horlick, L., and Havel, L.: Effect of Feeding Propylthiouracil and Cholesterol on the Blood Cholesterol and Arterial Intima in the Rat, J. Lab. & Clin. Med. **33**:1029, 1948.

50. Katz, L. N., and Stamler, J.: Experimental Atherosclerosis, Springfield, Ill., Charles C Thomas Publisher, 1952, p. 258.

51. Malinow, M. R.; Hojman, D., and Pellegrino, A. A.: Producción experimental de ateroesclerosis localizada en la rata, Rev. argent. cardiol. **19**:165, 1952.

52. Malinow, M. R.; Hojman, D., and Pellegrino, A.: Ateroesclerosis generalizada experimental en la rata, Ciencia e investig. **9**:39, 1953.

53. Malinow, M. R.; Hojman, D., and Pellegrino, A.: Different Methods for the Experimental Production of Generalized Atherosclerosis in the Rat, Acta cardiol. **9**:480, 1954.

54. Hartroft, W. S.; Ridout, J. H.; Sellers, E. A., and Best, C. H.: Atheromatous Changes in Aorta, Carotid and Coronary Arteries of Choline-Deficient Rats, Proc. Soc. Exper. Biol. & Med. **81**:384, 1952.

55. Wilgram, G. F., and Hartroft, W. S.: Pathogenesis of Fatty and Sclerotic Lesions in the Cardiovascular System of Choline-Deficient Rats, Brit. J. Exper. Path. **36**:298, 1955.

56. Wilgram, G. F.; Best, C. H., and Blumenstein, J.: Aggravating Effect of Cholesterol on Cardiovascular Changes in Choline Deficient Rats, Proc. Soc. Exper. Biol. & Med. **89**:476, 1955.

57. Bragdon, J. H., and Mickelsen, O.: Experimental Atherosclerosis in the Rat, Am. J. Path. **31**:965, 1955.

Mucus-Producing Cystadenocarcinoma of the Renal Pelvis and Ureter

Fourth Reported Case

JOHN A. ARCADI, M.D., Whittier, Calif.

In 1929 Plaut [1] recorded the first case of a mucus-producing adenocarcinoma of the renal pelvis. Since that time reports of two other cases have appeared in the literature.* The fourth such case is presented here.

CASE REPORT

A 68-year-old male sanatorium employee was admitted to the urological service of The Johns Hopkins Hospital on Aug. 3, 1954, because of severe right flank pain of two days' duration. The family and past general histories were noncontributory.

The present illness appears to have begun about 18 months prior to admission, when the patient had an episode of severe right flank pain lasting one or two days. Hematuria, lithuria, dysuria, or pyuria was not noted. He had had similar attacks, which spontaneously subsided, one year before admission, and also six months before admission. Within the six months preceding admission the patient had had several episodes of painless hematuria, which also subsided spontaneously.

Two days before his admission to the hospital the patient developed excruciating right flank pain, nausea, and vomiting. He had no urinary symptoms.

Physical examination revealed an emaciated, chronically and acutely ill 68-year-old white man. His blood pressure was 168/85 mm. Hg. Significant physical findings were limited to the abdomen. There was a well-healed right-lower-quadrant appendectomy scar. The right side of the abdomen was filled with a hard, tender mass that extended across the midline about 4 cm. The mass could be moved by palpation through the right flank. Good bowel sounds were heard on the left side, and none were heard over the mass on the right. There was no tenderness on the left side or in the left flank. The urine was loaded with pus cells, but was otherwise normal. The hemoglobin was 13.0 gm. per 100 cc., and the white blood cell count was 12,750 WBC/cu. mm. Blood chemistry values were within normal range.

Fig. 1.—Plain film of the abdomen showing both an x-ray and the actual calculi placed on the film. Note the gas-filled bowel displaced to the left by the large mass.

A plain film of the abdomen revealed a mass in the right abdomen associated with several large calculi (Fig. 1). The bowel was displaced to the left and appeared slightly distended. An intravenous pyelogram demonstrated no function in the right kidney and good function in the left, with a small stone in the superior pole of the left kidney. A chest film was normal.

On Aug. 4, cystoscopy was performed, and purulent-appearing material was seen pouring out of the

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* References 2 and 3.

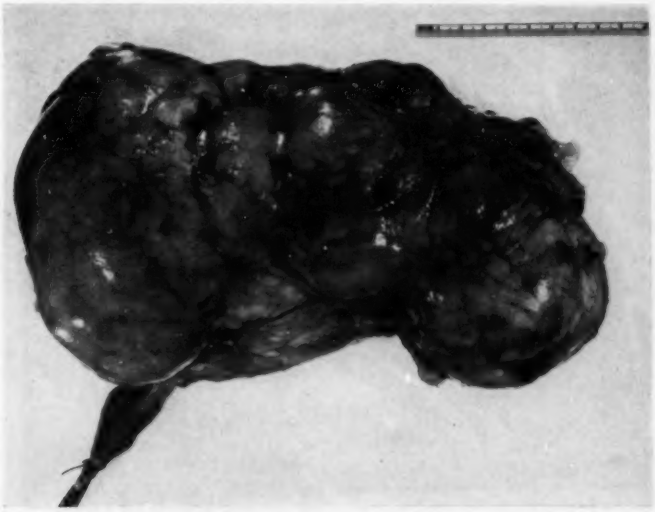


Fig. 2.—Gross specimen of the kidney with a portion of the ureter. The kidney is tense and lobulated.

right ureteral orifice. The left ureteral orifice was normal. There were no stones, tumors, or diverticula in the bladder. A catheter was passed up to the right kidney, but no drainage was obtained and nothing could be aspirated. Retrograde pyelography was not carried out.

A preoperative diagnosis of calculous pyonephrosis was made. On Aug. 6, through the beds of the 11th and 12th ribs, the kidney was removed extrapleurally and extraperitoneally. About 7 cm. of ureter was taken with the specimen. At no time was the renal substance or pelvis entered.

The patient's postoperative course was uneventful except for an episode of acute retention on the first postoperative night. About 400 ml. of thick gelatinous urine was removed by catheterization. He took fluids and food poorly until about the seventh postoperative day. After that he did well and was discharged on the 10th day after operation.

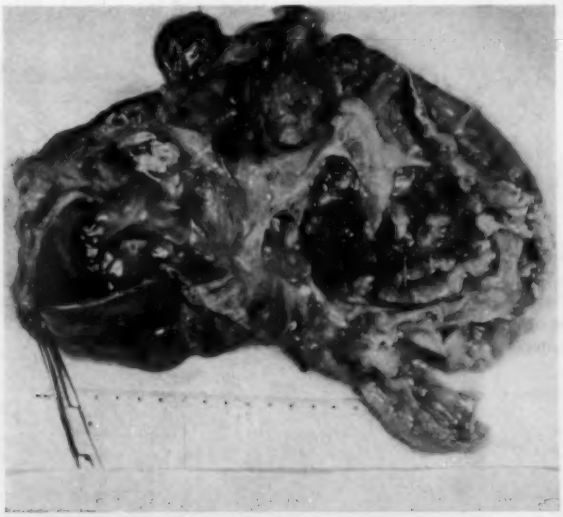


Fig. 3.—The kidney has been opened longitudinally showing calculi and shiny gelatinous material in the renal pelvis.

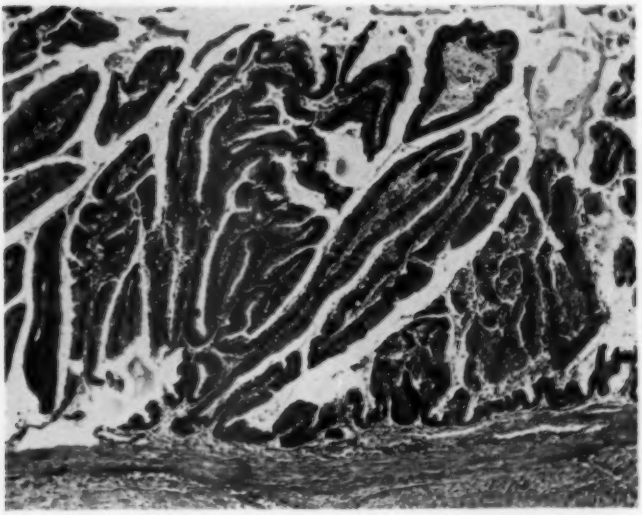


Fig. 4.—The papillary tumor with some glandular elements is seen. Tall columnar cells are seen in most of the tumor. Hematoxylin and eosin; reduced slightly from mag. × 50.

Pathological Report (Dr. James Shaka): The kidney weighed 2160 gm. (4.75 lb.) and measured 25 × 30 × 13 cm. (Fig. 2). Section through the kidney (Fig. 3) revealed a greatly dilated pelvis and calyces filled with an amber gelatinous material. Some calyces contained thick yellow pus. Several calculi (Fig. 1) were found in the pelvis and calyces. The renal parenchyma was reduced to a thin wall measuring 2 mm. in thickness.

In the pelvis there were several pink, papillary and granular masses projecting from the mucosal surface, measuring up to 1 cm. in diameter. Similar areas were seen in the ureter.

All sections of kidney and renal pelvis showed a loss of normal transitional epithelium and replacement by a papillary tumor composed of extremely tall columnar cells, some of which were arranged in glands of varying size (Fig. 4). The lumina of some of the glands and the gland cells contained mucus. The periodic acid-Schiff stain corroborated this observation (Figs. 5 and 6).

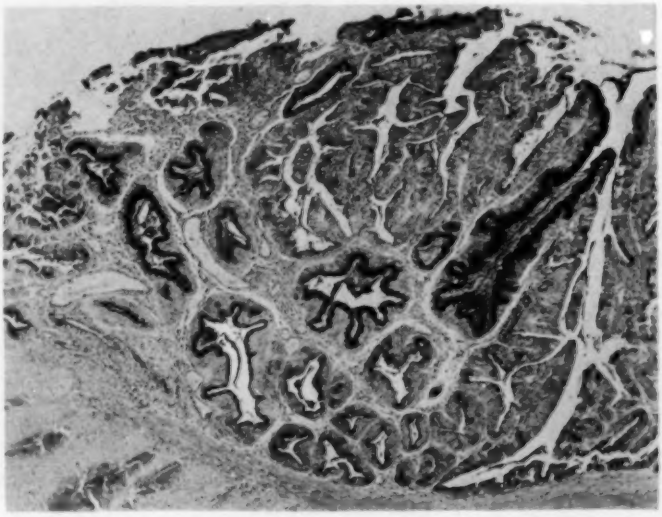


Fig. 5.—Section of tumor stained with the periodic acid-Schiff technique to demonstrate mucin; reduced slightly from mag. × 50.

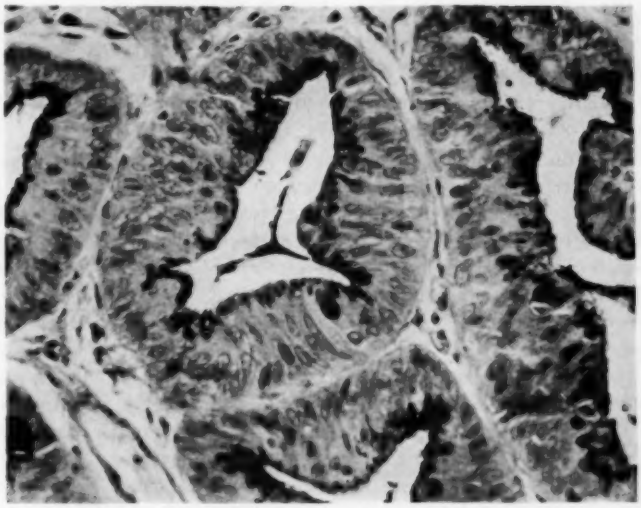


Fig. 6.—Same stain as in Figure 5, showing mucin in the secretory pole of the gland cell. Nuclear pleomorphism is also seen. Reduced slightly from mag. × 400.

The nuclei of the tumor cells were elongated, oval, and variable in their staining characteristics (Fig. 6). Foci of round cells were scattered throughout the renal parenchyma. Numerous cysts, seen in the renal parenchyma, were lined by the tumor cells described above. Sections of the ureter showed replacement of the normal transitional epithelium by the mucus-forming tumor (Fig. 7).

Diagnosis: Mucinous cystadenocarcinoma of renal pelvis.

The patient was advised to have a ureterectomy as soon as there was some improvement in his general health. Personal problems prevented him from returning for operation until Oct. 8, when a right ureterectomy was performed. The ureter was excised at the ureterovesical junction without a cuff of bladder. The postoperative course was uneventful, and he was discharged on the 11th postoperative day.

Pathological Report (Dr. Edward Andrews): The specimen of ureter measured 14 cm. in length. Seven raised granular nodules measuring 0.7 to 1.0

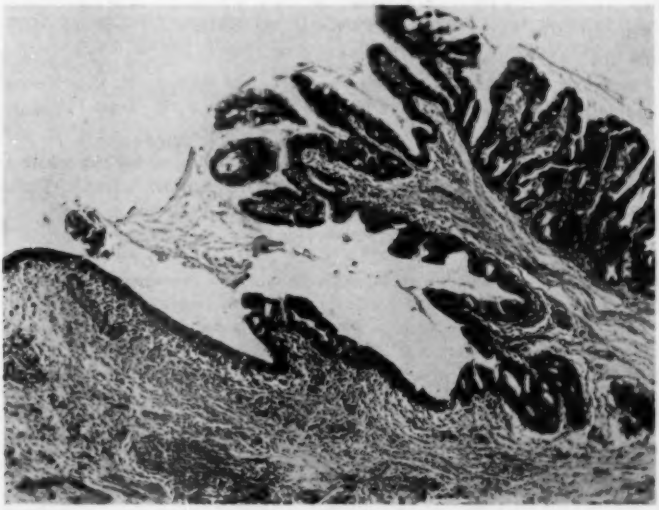


Fig. 7.—Tumor is seen arising adjacent to the normal epithelium of the ureter. The glandular and papillary components are well seen here. Hematoxylin and eosin; reduced slightly from mag. × 50.

cm. were seen along the mucosal surface of the ureter. No tumor was seen in the lower portion of the ureter near the point of transection.

Microscopically the ureter showed a transitional epithelium which could be followed into a mucus-forming adenocarcinoma. The tumor replaced the normal epithelium but did not appear to have penetrated the muscular wall of the ureter. In one or two areas small cysts were formed within the mucosa. This tumor was indistinguishable from that seen in the renal pelvis described above and presumably is metastatic to the ureter.

Diagnosis: Mucinous cystadenocarcinoma of the ureter.

The patient continued to do well, gain weight, and feel much stronger. Cystoscopy on Nov. 2 and Nov. 30 revealed mucoid material in the region of the right ureteral orifice. A transurethral resection of this orifice and the prostate was done on Dec. 14. The resection was carried down into muscle until a ureteral lumen was no longer seen. No tumor was found in the tissue removed, although many sections were cut. Some mucus was seen, but no abnormal cells were noted (Dr. William Reinhoff III). The mucus had probably come down into the intramural ureter from the tumors in the ureter and pelvis that were removed at an earlier date.

The patient was last heard from in December, 1955, at which time he was doing carpenter work and feeling well. Chest x-ray has remained normal.

COMMENT

In two † of the three previously reported cases, stones were associated with the mucin-producing tumor described. In the third case [2] the author presumes that there had been renal calculi present on the basis of the clinical history. He was not present when the kidney was removed. Our case likewise had many calculi in the diseased kidney. Ackerman,[2] as well as Ragins and Rolnick,[3] has suggested that this type of renal tumor arises by a metaplasia of normal epithelium induced by the stones and long-continued infection.

It appears more reasonable, to us, to postulate that the tumor arose *de novo* and that the thick gelatinous material secreted by it produced obstruction and stone formation. It has been suggested by Engel [4] that renal lithiasis may occur "through an interaction between glycoproteins and inorganic ions." Mucin contains a large amount of glycoprotein. With the great excess of mucin in the renal pelvis in these cases, stone formation could easily be explained on this basis.

† References 1 and 3.

SUMMARY

The fourth case of mucus-producing cystadenocarcinoma of the renal pelvis is reported. This case was treated by nephroureterectomy and transurethral resection of the ureteral orifice. The mucoid gelatinous secretion of the tumor may contribute significantly to the obstruction and to the formation of the stones.

REFERENCES

1. Plaut, A.: Diffuses dickdarmähnliches Adenom des Nierenbeckens mit geschwulstartiger Wucherung von Gefässmuskulatur, Ztschr. urol. Chir. **26**:562-578, 1929.

2. Ackerman, L. V.: Mucinous Adenocarcinoma of the Pelvis of the Kidney, J. Urol. **55**:36-45 (Jan.) 1946.

3. Ragins, A. B., and Rolnick, H. C.: Mucus Producing Adenocarcinoma of the Renal Pelvis, J. Urol. **63**:66-73 (Jan.) 1950.

4. Engel, M. B.: Mobilization of Mucoprotein by Parathyroid Extract, A. M. A. Arch. Path. **53**:339-351 (April) 1952.

News and Comment

ANNOUNCEMENTS

Fellowship in Cardiovascular Pathology.—A fellowship in cardiovascular pathology, established at the Mount Sinai Hospital of Greater Miami (4300 Alton Rd., Miama Beach 40) by the Bayshore Exchange Club of Miami Beach, offers a salary of $10,000 a year, with the expectation of continuance for two more years. Applicants must be eligible for the examinations of the American Board of Pathology or specialists certified by the Board. The research, which will be related to congenital heart disease, the conduction system, and the pathology of the arteries and veins, will be carried out on a full-time basis in the research laboratories of the Mount Sinai Hospital under the direction of Dr. Maurice Lev.

SOCIETY NEWS

Annual Meeting of American Institute of Dental Medicine.—The Thirteenth Annual Meeting of the American Institute of Dental Medicine will be held at El Mirador, Palm Springs, Calif., Nov. 4 to 8. Applications and full information may be secured from the Executive Secretary, Miss Marion G. Lewis, 2240 Channing Way, Berkeley 4, Calif.

Third National Cancer Conference.—The American Cancer Society and the National Cancer Institute of the Public Health Service, Department of Health, Education, and Welfare, will jointly sponsor the Third National Cancer Conference in Detroit, June 4, 5, and 6.

The opening session of the conference, in the Sheraton-Cadillac Hotel, will feature addresses by Dr. John R. Heller, Director of the National Cancer Institute, and Dr. Charles S. Cameron, Medical and Scientific Director of the American Cancer Society. Morning and afternoon sessions of the three-day meeting will begin with a general session at which a subject of broad interest will be presented by an outstanding speaker. The general sessions will then break into various symposia to discuss cancer of different body sites, such as lung, gastrointestinal tract, and breast.

A Host Committee representing medical and health groups of the Detroit area, headed by Dr. Harry M. Nelson, of the Southeastern Michigan Division, American Cancer Society, will arrange a public Cancer Forum for Tuesday evening, June 5, as a special feature of the conference. Speakers for this program will include several participants in the scientific program of the Cancer Conference.

Copies of the conference program and advance registration cards may be obtained from the National Cancer Conferences Coordinator, American Cancer Society, 521 W. 57th St., New York 19.

All physicians are invited to attend.

Medicolegal Workshop.—A Medicolegal Workshop for medical examiners, pathologists, and physicians is to be given at the Medical College of Virginia, Richmond, Va., on Friday, March 23. The program is sponsored by the Department of Legal Medicine, Medical College of Virginia, the Chief Medical Examiner's Office, State Department of Health, and the Virginia Society of Pathology and Laboratory Medicine. The registration fee is Twenty-five Dollars.

International Society of Hematology.—The International Society of Hematology will hold its sixth congress in Boston, Aug. 26-Sept. 1. The meetings will be held at the Hotel Somerset and will include discussions on leukemia, nucleonics, spleen and reticuloendothelial system, hemorrhagic disorders, anemia, and immunohematology (autotype). Further information can be obtained by writing to Dr. James L. Tullis, New England Center Hospital, Harrison Ave. and Bennet St., Boston 11.

Books

Cardiac Diagnosis: A Physiologic Approach. By Robert F. Rushmer, M.D. Price, $11.50. Pp. 447, with illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1955.

This book is one which should be read and kept on the reference shelf of physicians, physiologists, and pathologists who are interested in the heart. The text is well written in a logical manner, so that a person who is starting in this field can learn with ease. On the other hand, sections which occur later in the book are presented as finite entities which are not completely dependent upon earlier chapters. This latter fact makes the book valuable for reference purposes.

The many illustrations are excellent, including those from Dr. Rushmer's illuminating experimental work on cinefluorographic angiocardiography. The drawings are clear and augment the text in those places where both photographs and words would fail. Some of those associated with the presentation of electrocardiography in particular are unique.

The extensive bibliography at the end of each section should be helpful to people who are interested in searching the literature in the area of the physiology and pathology of the cardiovascular system.

All in all, this book is a good one.

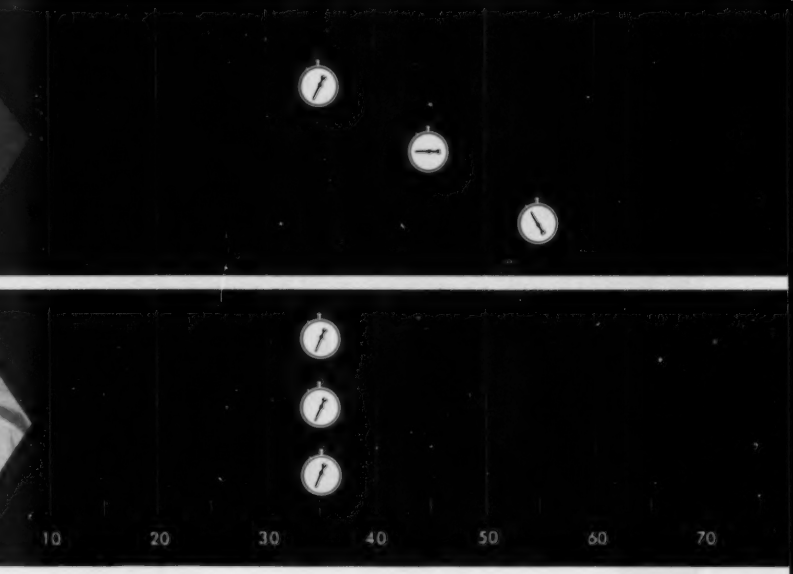
Perinatal Mortality in New York City: Responsible Factors: A Study of Nine Hundred Fifty-Five Deaths. By the Subcommittee on Neonatal Mortality of the Committee on Public Health Relations, New York Academy of Medicine. Analyzed and reported by Schuyler G. Kohl, M.D., Dr. Ph. Price, $2.50. Pp. 112, with 67 tables. Harvard University Press, Cambridge 38, Mass., 1955.

This study deals with an attempt to assess the causes of death in premature and mature infants. Dr. M. Chandler Foot served as director of the study. Dr. Schuyler G. Kohl did the major assembling and analysis of the data, and Dr. John W. Fertig served as consultant on statistics. Nine hundred fifty-five cases were chosen for the study, which was conducted by a committee of the New York Academy of Medicine. The book is of interest from several standpoints: It illustrates a specific version of the form such a study may take. The difficulties in collection of data are illustrated. For example, only thirty-five per cent of the infants were brought to autopsy. Interesting are the figures on lack of agreement of the death certificate diagnosis, clinical diagnosis, and autopsy diagnosis: With complete autopsy the death certificate and anatomical diagnosis agreed only forty per cent of the time; the clinical diagnosis was in agreement with the autopsy findings sixty-seven per cent of the time; in all nine hundred fifty-five cases the death certificate agreed with the clinical diagnosis only forty-four per cent of the time. As the authors recognize, it would be advisable to have complete autopsies on all infants performed in a uniform manner and oriented to the particular problems of such a study. The main purpose of the study was to determine how many "perinatal" deaths are preventable. From the totality of clinical, interview, and autopsy material, it was decided that thirty-five per cent of the deaths among the premature and sixty-two per cent of those among the mature infants were preventable. It is evident from this report that, as in the past so in the future, the pathologist must play an important role in public health and preventive medicine.

Ion Exchange and Adsorption Agents in Medicine. By Gustav J. Martin, Sc.D. Price, $7.50. Pp. 333, with 26 illustrations. Little, Brown & Company, 34 Beacon St., Boston 6, 1955.

The author in this volume attempts to restore interest in the old theory of intestinal stasis with autointoxication. It is his contention that all chronic degenerative disease has as an important component in its etiology the absorption from the intestine of small quantities of toxic chemicals. These agents produce imperceptible but irreversible changes in tissues and in the course of years create gross pathology. He believes that the absorption of these toxic agents can be prevented by proper selection of ion exchange and adsorption materials.

in prothrombin time determinations



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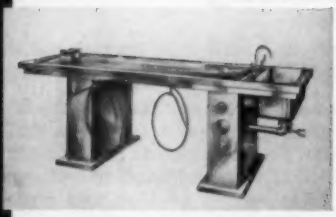

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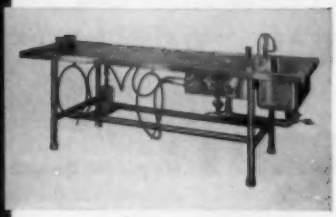

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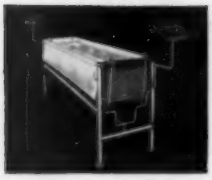

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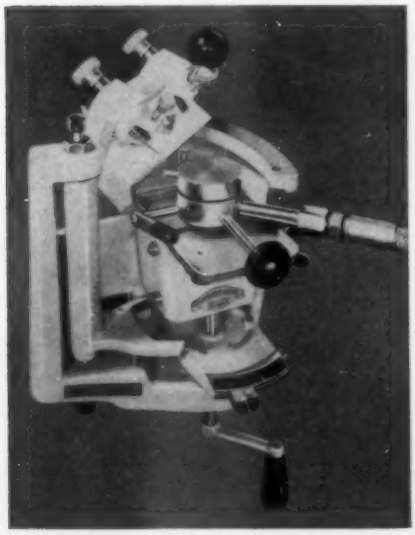

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